

Investigating the rate of fungal growth during the latent period of *Zymoseptoria tritici* in winter wheat cultivars

Thesis submitted in fulfilment with Degree of Master of Science by Research (MSc(R))

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Abstract

Winter wheat is one of the most important tillage crops in Ireland and *Septoria tritici* blotch (STB) resistance is a key beneficial trait for wheat grown there, owing to a climate that facilitates high disease pressure, its impact on crop yield and associated costs of protecting crops against disease *Zymoseptoria tritici*, the causative pathogen of STB grows inside host tissues for typically 10-14 days before disease symptoms become visible, beginning with chlorosis of the leaf, then progressing to necrosis and finally small black pycnidia appear. Little fungal biomass accumulates during this latent period (LP), with large increases in fungal growth visible as the fungus shifts into the necrotrophic stage. However, a steady increase in *Z. tritici* DNA during the LP has been detected, using quantitative PCR. Throughout the course of this research the LP was defined as the time from infection by *Z. tritici* to the appearance of pycnidia on the leaf surface. Through a combination of laboratory, glasshouse and field experimentation in Ireland and England, this research provided a new insight into the LP of *Z. tritici*, by identifying a wide range of LP among diverse cultivars in glasshouse conditions [Chapter 2], substantial variation in LP and the rate of disease progression between seven cultivars in field conditions [Chapter 3] and the high correlation between Cq values, fungal growth and disease symptoms, indicating the potential for qPCR to be used to estimate the LP of STB [Chapter 4].

Latent periods vary between cultivars and knowledge of the length of the LP assists in identifying the factors which cause infection. Critically, the length of the LP is an important epidemiological factor in subsequent pathogen spread and the generation of epidemics. Therefore, identifying cultivars with extended LP's will in future increase opportunities to develop STB resistance in winter wheat populations.

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Signed: 

Date: 1st December 2017

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Abbreviations

ANOVA: Analysis of variance

Avr: avirulence

AWS: Automatic Weather Station

bp: base pair

cv: cultivar

cvs: cultivars

Cq: quantitative cycle

DAFM: Department of Agriculture, Food and the Marine

Dai: Days after inoculation

DNA: Deoxyribonucleic acid

DMI: Demethylation inhibitor

EU: European Union

FP: first appearance of pycnidia

GS: Growth stage

LP: Latent period

LSD: Least significant difference

MBC: Methyl benzimidazole carbamate

PCR: Polymerase chain reaction

PDA: Potato dextrose agar

qPCR: Quantitative polymerase chain reaction

QoI: Quinone outside inhibitor

R: resistance

RNA: Ribonucleic acid

SDHI: Succinate dehydrogenase inhibitor

STB: Septoria tritici blotch

TCA: tricarboxylic acid

UK: United Kingdom

Chapter 1: Introduction

1.1 Preface

Wheat is amongst the main staple crops in the world alongside maize and rice (Jones, 1978). It is the most widely cultivated cereal in the world, in 2013–2014 harvests reached 705 million metric tonnes (www.agrimoney.com, 2015).

Advances in crop breeding have led to increased quality and yield of wheat crops, and thanks to improvements in management and mechanisation, production has become more efficient than in the past. Wheat provides 20% of daily protein and calories for human consumption; it is rich in carbohydrates (mainly starch) for energy (80% content), protein (gluten) (9-15% content), essential vitamins and minerals. Wheat is also one of the few crops which is adapted to low temperatures during the cool season (Jones, 1978).

The world population is expected to reach 9 billion by 2050 and demand on wheat is expected to increase by 70% (O'Driscoll *et al.*, 2014). This will bring with it a challenge for the world food production industry in finding a way to produce more food to feed a growing population using the existing agricultural land base, while preserving biodiversity, reducing greenhouse gases, safeguarding the environment and adapting to climate change. Adding to the existing land base is not a feasible option, as this would lead to an increase in deforestation.

Therefore the only option is to grow more food on the existing land base or to eat less meat from intensively-reared animals fed on grain. This can be achieved by growing higher yielding crops, with an increased resistance to disease and pests.

The Global Wheat Initiative was launched in 2011, with a goal of co-ordinating global wheat research. Through this international effort, the progress needed to increase wheat production, quality and sustainability can be achieved, thus contributing to the global efforts towards food security and safety under changing climate conditions. Annual yields will need to increase from the current level of below 1% to at least 1.7% (Wheatinitiative.org, 2015). Ireland

and the UK are two of the top four countries in the world for wheat yields per hectare (Figure 1.1). At present, without disease management and crop protection approximately 50% of the wheat crop would be lost to diseases, comprising of fungal and bacterial pathogens (16%), viruses (3%), animal pests (9%) and weeds (23%). Using the best techniques available (soil preparation, crop rotation, fungicides, pesticides, herbicides, and advanced harvesting methods), this can be reduced to a loss of approximately 30% (10% to pathogens, 2% to viruses, 8% to animal pests and 9% to weeds) of the crop to pests and disease. This gives plenty of room for innovation and improvement (foodsupplychallenges.eu, 2015). These figures are based on Europe (Oerke & Dehne, 2004).

The research which is carried out by Teagasc, Ireland and The John Innes Centre, Norwich, UK, can potentially provide today's farmers with the resources they need to ensure sustainable food production.

Top 20 wheat yields (t/ha) by country (2011)

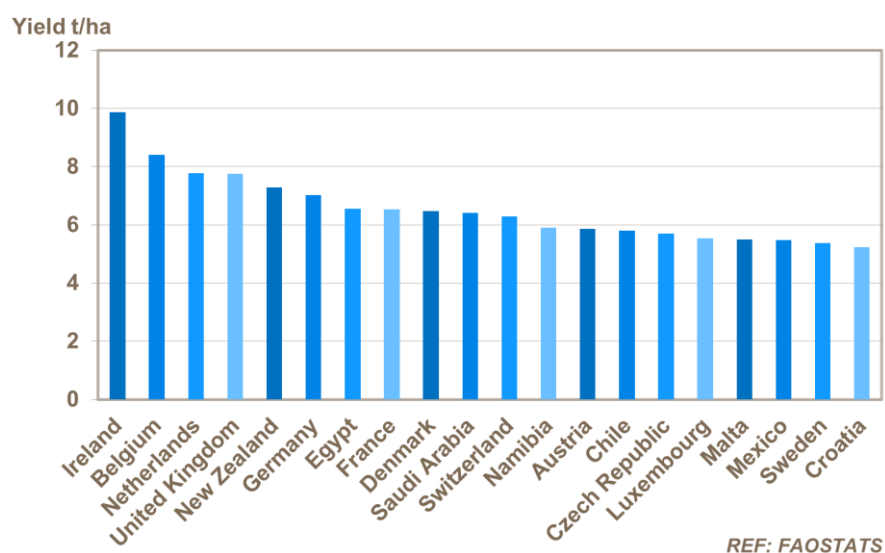


Figure 1.1: Top 20 wheat yielding countries in the world in 2011 (fao.org/worldfoodsituation/en/, 2015).

1.1.1 The importance of wheat as an EU crop

Wheat is the most important crop within the Food and Agriculture Organisation of the United Nations region of Europe and Central Asia. This region accounted for 40% of the world wheat production in 2011, with the EU producing just over half of the region's wheat crop. France is the EU's top wheat producing country, harvesting 38 million tonnes from 5.8 million ha – a yield of 6.5 tonnes per hectare (faostatisticalyearbook, 2014). Although Ireland's total wheat production is small compared to e.g. France, Germany, Ukraine and England, the yields in south-eastern counties are among the highest in the world (Burke *et al.*, 2011). In 2013/2014 the EU produced over 143 million metric tonnes (mmt) of wheat; 60% more than USA and 35% more than India (fao.org/worldfoodsituation/en/, 2015). Europe's wheat crop is of significant economic and social importance (Fones & Gurr, 2015); up to 15% of the EU's harvest is currently exported. Nearly two-thirds of the EU's cereals are used for animal feed, around one-third for human consumption and only 3% is used for biofuels, (ec.europa.eu/agriculture/cereal, 2015).

The EU's wheat crop is worth several billion Euros annually. The value of the wheat harvest to the individual economies is significant. In 2013 the harvests of the three main wheat growing countries within the EU were worth €7.2 billion to France, €4.9 billion to Germany and €2.4 billion to the UK.

1.1.2 Wheat production in Ireland

Ireland produces approximately 700,000 tonnes of winter wheat annually. However, in 2013 production decreased by 32.5% (204,000 tonnes) this was due to a decrease of 46.3% (39,200 hectares) in the area sown (CSO, 2014). Two thirds of the wheat grown in Ireland is used for animal feed; however the remainder is a valuable raw material for Ireland's brewing, distilling and flour milling industries. Large industrial bakers tend to get their wheat from France and Canada, as a dry climate is necessary to produce hard wheat's, which are desirable in the baking industry. This is due to the fact, that the majority of wheat grown in Ireland is winter-sown wheat (in October), not as high in its gluten content as spring-sown wheat (in April) which is more suitable for flour production. Ireland imports nearly 700,000 tonnes of wheat annually. Economic sustainability of winter wheat production in Ireland is under increasing pressure

due to the variability in grain prices and also stagnation of yearly wheat yields (Lynch *et al.*, 2017), which had until recently seen a steady increase since the 1960's (CSO,2015).

There are approx. five to eight different varieties of winter wheat sown in Ireland each year and growers tend to sow varieties which feature on the Department of Agriculture (DAFM) National Recommended Lists. These varieties are recommended due to their yield potential, resistance to disease and grain quality (DAFM, 2015).

1.2 Septoria tritici blotch

1.2.1 Importance / Impact of Septoria in wheat

Wheat is susceptible to multiple diseases, including Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*). STB occurs throughout the world in countries including the United States, South America, parts of Australia, Mediterranean region and many parts of Europe including Ireland. Severe epidemics of STB can reduce winter wheat yields by up to 50% in fields where susceptible wheat varieties were planted and in climates conducive to disease (Fones & Gurr, 2015). STB is one of the most devastating foliar diseases of autumn sown winter wheat (*Triticum aestivum*) crops in Europe (Dean, 2012; Fones, 2015) and requires intensive chemical control measures to protect yields. Approximately 70% of Europe's annual cereal fungicide input is used to control STB (O'Driscoll *et al.*, 2014). It is among one of the top most economically damaging diseases of this crop in Ireland, giving rise to a focus of intense research across the primary cereal producing countries of the world. The severity of this disease depends on disease pressure, which is a measure of disease inoculum present in the environment along with weather and region. The presence of wheat stubble and debris, volunteer wheat and susceptible grasses are main attributes to the survival of the disease from year to year (Duveiller, 2009). The pathogen must remain present on the plant wheat stubble and debris in the form of survival structures (ascospores, pycnidiospores, mycelium) to produce primary inoculum

at seedling emergence, thus surviving the intercrop period (Suffert *et al.*, 2011). Rainfall during stem extension assists in the spread of the disease into the upper leaves of the canopy (Royle *et al.*, 1986). Yield losses occur when the top 2 or 3 leaves, which are important contributors to grain filling, become infected with STB (Thomas, 1989).

To control the levels of disease in these top leaves, either host resistance must be developed or a fungicide application programme put in place. Some varieties of winter wheat are highly resistant to other diseases, such as yellow rust, but not to STB, however if disease pressure is low fungicide treatments may not be required. Other varieties are highly susceptible and will require high fungicide input to reach their yield potential (HGCA, 2013). STB resistance is known to be a key beneficial trait for wheat grown in Ireland, due to a climate of mild winter temperatures and high rainfall that facilitates rapid dispersal of splash borne spores and disease development (O'Driscoll *et al.*, 2014). It has been reported that wheat has two types of resistance to STB, Qualitative resistance and Quantitative resistance (Brown *et al.*, 2015). By combining 1. estimation of losses caused by STB and 2. understanding the host-pathogen relationship in a certain environment, it will help in selecting an appropriate disease management strategy (Goodwin, 2007).

1.2.2 Biology of *Zymoseptoria tritici*

Z. tritici has been present in wheat since host domestication 10,000 – 12,000 years ago in the Fertile Crescent (a region of moist and fertile land which includes countries such as Iraq, Syria, Israel, Cyprus and Egypt). The wheat infecting pathogen emerged from closely related *Mycosphaerella* pathogens infecting wild grasses. *Z. tritici* co-evolved and has spread with its host globally (Stuckenbrock *et al.*, 2010). It is the causative agent of STB characterised by chlorosis, necrotic lesions and pycnidia within the lesions on the leaves and is most prevalent in cool, wet conditions, so Ireland has an ideal climate for its growth. Propagated by both sexual ascospores and asexual pycnidiospores, the first visual symptom of STB is chlorosis of the infected leaf. As the disease develops within the leaf, necrotic lesions start to appear. These necrotic lesions

will then start to display small black fruiting bodies called pycnidia (Cohen & Eyal, 1993).

The vegetative growth forms of *Z. tritici* fall into three categories (i) Single-celled yeasts (Nicolaisen *et al.*, 2009), (ii) Multi-cellular and (iii) Tip growing hyphae. The most common cell type is the macroconidium, which is often referred to as the “yeast-like” stage. These cell types can be seen when grown under laboratory conditions (Steinberg, 2015). Macropycnidiospores are multi-cellular, whereas yeasts are uni-cellular. However *Z. tritici* also produce micropycnidiospores, which are small and uni-cellular structures, as reported by Eyal, 1987. Therefore, they may fall into the definition of a “yeast-like” growth form. The micropycnidiospores are formed by lateral budding of the hyphae or macropycnidiospores. Finally, macropycnidiospores germinate to form thin hyphae, consisting of very elongated cells that extend by polar tip growth, which puts them in the third category of tip growing hyphae. Many pathogenic fungi have the ability to grow vegetatively in several growth forms, with hyphal growth being a prerequisite for invasion of host tissue (Steinberg, 2015; Cohen & Eyal, 1993; Eyal, 1987).

The biphasic infection process of *Z. tritici* differs from other pathogenic fungi. Biotrophic fungi such as *Blumeria graminis*, the causative pathogen of powdery mildew, require a living host to complete their life-cycle; others rapidly kill their host to acquire nutrients for growth such as *Botrytis cinerea*, the causative agent of bunch rot in grapes. *Z. tritici* begins its lifecycle as an endophyte within living host tissue; if it feeds at all from its host, which is uncertain, it derives its nutrition from the apoplast around living cells (Orton *et al.*, 2011). This early phase is asymptomatic and is a ‘latent period’, where the fungus grows inside the host with no symptom expression. To complete its life cycle *Z. tritici* kills the surrounding host cells and switches to a necrotroph meaning it utilizes dead tissue as its source of nutrition. What molecular and environmental triggers are responsible for this trophic switch remain unknown. It has been suggested that environmental stresses such as light, water availability and temperature fluctuations may play a role, but it may also be linked to host developmental signals or pathogen conditions (Sanchez-Vallet *et al.*, 2015).

1.2.3 The infection cycle

There are two types of spore which cause the dispersal of *Z. tritici* inoculum in the environment: (i) air-borne sexual ascospores which can be wind-dispersed over hundreds of kilometres (Nicolaisen *et al.*, 2009) and (ii) rain splash dispersed asexual pycnidiospores which are dispersed more locally and are the causative agents of infection. A pool of pycnidiospores are produced within the pycnidia and then released sequentially, according to rain events. The production of pycnidiospores has been reported to peak after the first rainfall, with fewer and fewer pycnidiospores being released after each subsequent rainfall, reviewed in (Suffert *et al.*, 2011). Primary infection occurs soon after seedlings emerge either in the winter (for winter wheat) or the spring (for spring wheat) by air-borne ascospores, which are present on crop debris and volunteer hosts. There are not as many ascospores present in the environment in the spring, therefore infection is generally lighter on spring crops. Secondary infection occurs when the asexual pycnidiospores are dispersed throughout the leaf canopy and to other plants by direct contact and rain-splash (Suffert *et al.*, 2011). Optimum environmental conditions for the infection process to occur are wet, cloudy days with temperatures between 20 and 25°C. However, infection can occur at temperatures as low as 2-3°C and as high as 33-37°C. Lower temperatures of approx. 4°C will increase the time required for spore germination, mycelial growth and lesion and pycnidia development (Palmer & Skinner, 2002; Eyal, 1987). It has also been reported that *Z. tritici* requires 6-24 hours of moisture for infection to occur, this moisture source could arise from rain, significant dew or high humidity within the canopy (Burrows, 2013). The infection process has been divided into three stages (i) entry into the host, (Nicolaisen *et al.*, 2009) (ii) colonisation of the host and (iii) formation of pycnidia (Steinberg, 2015).

Entry into the host occurs after the air-borne ascospores land on the leaf surface. The ascospores germ tubes are often branched and developed into clusters of hyphae in the stomatal depressions of the leaves, which has been suggested by Kema *et al.*, (1996) to be a random process. However, it has also been suggested that the growth of the hyphae towards the stomata is due to an unknown “thigmotropic signal” (Duncan & Howard, 2000). Penetration of the

leaf is strictly through stomata, to gain entry into the sub-stomatal cavity. Mycelium starts to appear after 12-24 hours in the sub-stomatal cavity. After 48 hours the fungal hyphae continue to inter-cellularly colonise the mesophyll, this usually occurs in close contact with the cell walls (Kema *et al.*, 1996b). Previous findings by Cohen and Eyal, (1993) suggest by 24 hours after inoculation of ten day old seedlings with a mixed *Z. tritici* isolate inoculum, 85 – 90% of conidia had germinated regardless of wheat cultivar (resistant or susceptible) and by 48 hours after inoculation, the germination rate had further increased and hyphae were observed. More recent research supports these findings in that once the pathogen is inside the leaf, *Z. tritici* colonizes the mesophyll tissue in an intercellular manner, but there is no evidence that feeding structures such as haustoria are produced (Palmer, 2002).

Colonisation of the host occurs about 3-11 days after infection when hyphae begin to fill the sub-stomatal space and pre-pycnidia appear in these cavities (Shetty *et al.*, 2003; Kema *et al.*, 1996b). As the asexual fruiting bodies (pre-pycnidia) develop, chlorotic lesions start to appear, these are then followed by necrotic areas ~ 10-12 days post-infection. It is the formation of these pre-pycnidia which marks the transition from the endophytic phase (latent phase) to the necrotrophic phase (Steinberg, 2015). This was also previously reported by Cohen and Eyal, (1993) where, by 12 days after inoculation, fungal tissue had occupied the stomatal chamber and also the intercellular spaces of the mesophyll tissue. Finally, this results in the formation of pycnidia which are solely restricted to the sub-stomatal spaces (“linear arrangement of pycnidia follows the linear pattern of stomata”) in both the partially resistant and the susceptible cultivars (Cohen & Eyal, 1993). These matured pycnidia produce the multi-cellular macropycnidiospores that develop from conidiospores (Kema *et al.*, 1996b).

Biomass of the fungus has been reported to insignificantly increase during the initial days of infection (Keon *et al.*, 2007; Shetty *et al.*, 2007; Ponomarenko & Kema, 2011). During the necrotrophic phase, the fungus appears less reliant on secreted proteases and displays cell wall attacking capabilities. Recent research has indicated the primary source of nutrients in the early phase of infection are released via β -oxidation of fatty acids and lipids and the activity of

hydrolytic enzymes such as plant cell wall degrading enzymes and peptidases (Rudd *et al.*, 2015; Steinberg, 2015).

Many cycles of asexual reproduction during the growing season, but only one cycle of sexual reproduction each year, allow epidemics to develop rapidly (Figure 1.2) (Suffert *et al.*, 2011; HGCA, 2016). The infection process of *Z.tritici* has been under investigation since the 1990s, but its trophic relationship with wheat remains controversial (Sanchez-Vallet *et al.*, 2015). How the switch from biotrophic to necrotrophic growth is triggered and also where the fungus obtains its nutrients allowing its growth during the long asymptomatic phase remains unclear.

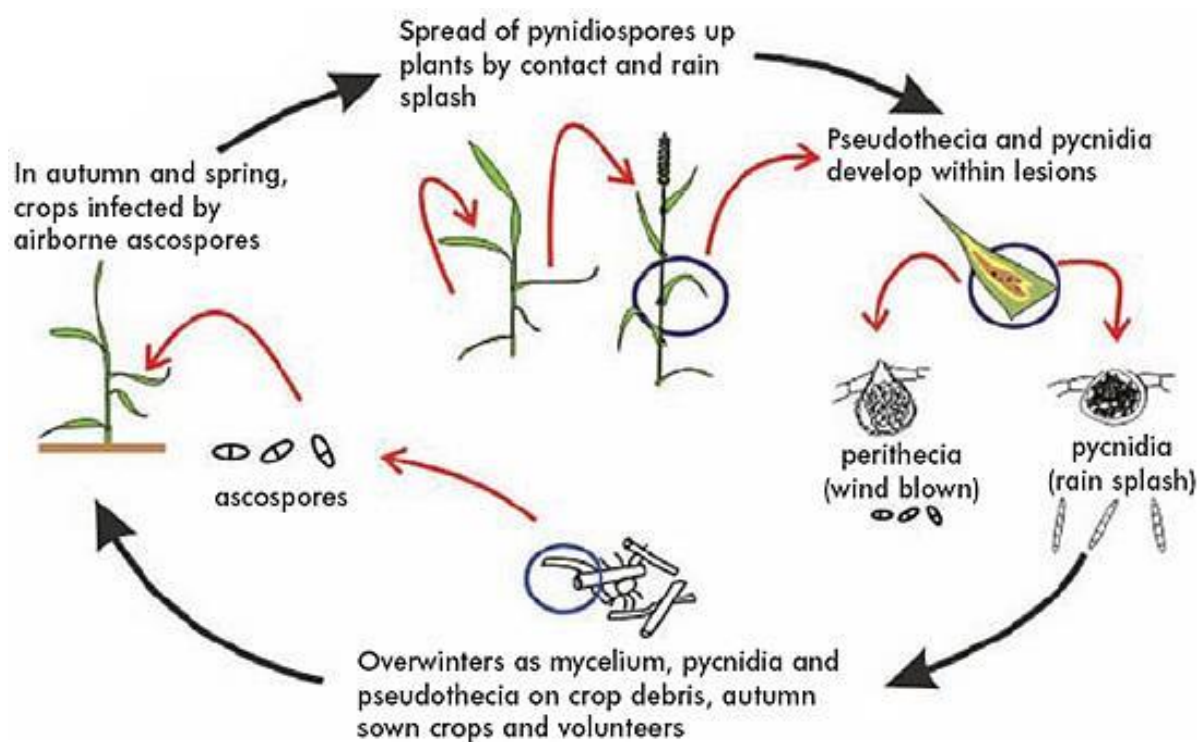


Figure 1.2: Infection process of *Zymoseptoria tritici* (HGCA, 2016; Suffert *et al.*, 2011)



Figure 1.3: Winter wheat cv. Gallant displaying pycnidia 27 days after inoculation with *Zymoseptoria tritici* Dutch Isolate IPO323. (Picture taken from Stigg and Gallant time course trial in glasshouse).

1.2.4 Latent Period

Zymoseptoria tritici infection remains asymptomatic during the first ~ 1-15 days, this is the 'Latent Phase', which can vary between 6 and 36 days depending on the wheat cultivar – fungal isolate combination and also the age of the leaf and the environmental conditions (Lee *et al.*, 2013). The length of the LP was previously reported by Viljanen-Rollinson *et al.*, (2005) to range between 14-21 days at an optimum temperature of 15-20°C and can be lengthened to approximately 40 days at a temperature of 5°C, depending on the cultivar and environmental conditions such as temperature and leaf wetness (Viljanen-Rollinson *et al.*, 2005). This suggests that the LP is directly affected by environmental conditions such as temperature, humidity and inoculum pressure. It has been reported that during the LP, the growth of *Z. tritici* is slower in a partially resistant winter wheat cultivar (Kema *et al.*, 1996b). After the initial infection of the leaf, the fungus grows within the leaf but is still exhibiting no visual symptoms (Figure 1.4). Similar studies into the length of the latent period have also shown wide variation, ranging from 14-21 days as observed by Eyal *et al.*, (1987), to 17-21 days as reported by Shaw *et al.*, (1990), to 21-27 as observed Armour *et al.*, (2004).

Knowing the latent period length under different environmental conditions, could assist in predicting *Z. tritici* events and the timing of fungicide spraying, it could also assist in applications to breeding for resistance and selection of resistant varieties, by allowing resistance genes to be isolated and their functions investigated.

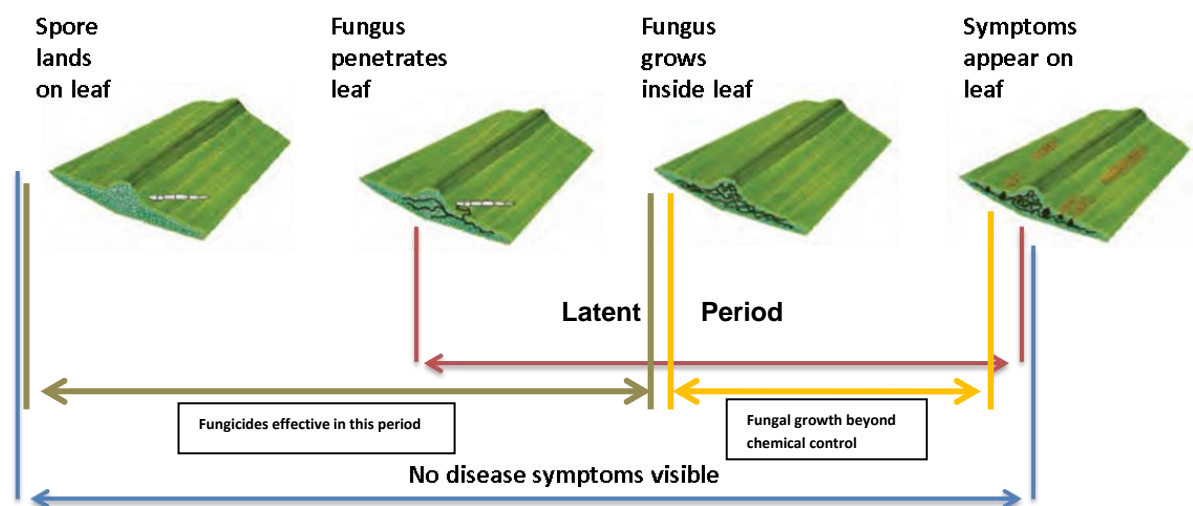


Figure 1.4: Latent period of *Zymoseptoria tritici* taken from the HGCA, Wheat disease management guide, Spring 2016, (HGCA, 2016).

1.3 Genetics of resistance to *Zymoseptoria tritici*

Wheat carries two types of resistance to STB; this was shown in a study of 238 wheat cultivars in the UK in the 1990's (Arraiano & Brown, 2006). The first type of resistance is *Qualitative resistance* also known as major gene resistance, usually has a large effect controlled by major genes. This type of resistance follows a gene for gene relationship, meaning the hosts and parasites ability to cause disease is controlled by pairs of matching genes, i.e. for every gene conferring resistance (R gene) in the host plant there is a corresponding gene for avirulence (Avr gene) in the pathogen. This implies that the resistance gene will be overcome once the pathogen acquires virulence either by the loss or the alteration of the corresponding gene for avirulence. This relationship has been demonstrated for cvs Flame and Hereward, where the resistant gene Stb6 (Brading *et al.*, 2002) was identified in the host plants and conferred resistance to the Dutch *Z. tritici* isolate IPO323.

The second type of resistance is *Quantitative resistance* also known as multiple gene resistance, which usually has a moderate to small effect controlled by several genes. This type of resistance is in many instances effective against all *Z. tritici* genotypes (Brown *et al.*, 2015).

Expression of quantitative or partial resistance may depend on the growth stage of the wheat plant (Chartrain *et al.*, 2004), whereas many genotype – specific qualitative resistances are independent of growth stage (Kema & van Silfhout, 1997). An example of the expression of a gene with a quantitative effect on disease is Stb17 in adult plants but not in seedlings (Tabib Ghaffary, 2012).

The genetic analysis of *Z.tritici* – resistance requires a scoring method of the phenotype, in most analyses the phenotype scored is the formation of pycnidia within the necrotic leaf tissue (Brown *et al.*, 2015). Whole seedling (Brading *et al.*, 2002) and detached leaf assays (Arraiano *et al.*, 2001a) are two tests which can be conducted at the seedling stage, with artificial inoculation taking place when seedlings are two weeks old, both tests require conditions of high relative humidity. Alternatively plants can be tested at the adult stage either in a glasshouse (Adhikari *et al.*, 2004b) or in a field environment (Kema & van Silfhout, 1997) both by artificial inoculation. In the field environment plants must be inoculated with a *Z.tritici* isolate at a dose which is sufficient to make contamination by natural infection comparatively negligible. When genetic analysis of naturally infected trials is conducted, resistance genes which are identified are effective against the current local *Z.tritici* population (Brown *et al.*, 2015).

1.4 Disease management and resistance to fungicides

1.4.1 Disease management

Growing wheat in Ireland can be a problem due to the wheat's susceptibility to fungal disease (*Septoria*, powdery mildew, Yellow rust, and eyespot), for this reason high inputs of fungicides and herbicides are required. Fungicide application to control disease is a major cost element in cereal production especially for winter wheat. The estimated cost of fungicide application for 2015 in Ireland is 190 €/ha (Teagasc, 2015). Reducing fungicide inputs, while maintaining yields will help improve the profitability of winter wheat production in Ireland.

There are several fungicides currently in use to control STB. In regions where the seedling stage can be affected by the pathogen, triazole fungicides can be used. The most common type of fungicide treatment is foliar sprays. It is only recommended to use fungicides when they are of economic benefit, taking into account the projected yield and loss from STB. Timing fungicide applications to periods when the pathogen is most likely to be active will give the best economic return. Foliar fungicides provide the best protection against STB when applied to the top three leaves of the wheat plant. It is these leaves which are the most important contributors to yield (Shaw & Royle, 1989). As soon as leaf three has just emerged (approximately GS 32) the first fungicide spray should be applied, this is referred to as T1, this aims to provide full protection to leaf three and also some protection to leaf two. When the flag leaf (leaf one) is just emerging (approximately GS 39), the second fungicide spray should be applied (HGCA, 2016) this is referred to as T2 and aims to eradicate any disease on leaf two and also protect the flag leaf from disease (Sylvester-Bradley *et al.*, 2008).

1.4.2 Resistance to fungicides

Fungicide resistance as defined by Anderson, (2005) occurs when a fungal pathogen survives and reproduces in the presence of a fungicide (Anderson, 2005). This acquired resistance occurs when a pathogen population which was initially sensitive to the fungicide at the time of introduction becomes insensitive over time and can no longer be adequately controlled. Fungicides which target multiple genes are considered to be at a lower risk of resistance than fungicides which target a single gene (Brent & Hollomon, 2007).

Resistance started to develop in the early 1970s to the broad spectrum Methyl Benzimidazole Carbamates (MBC) fungicides (commercially available since the 1960s) (O'Sullivan, 2009). These fungicides have systemic properties (the fungicide enters the plant and is transported around the plant by the transpiration stream (Russell, 2005)). MBCs impact on mitosis and normal cell division in target fungi; they have inhibitory effects on the biosynthesis of tubulin into microtubules. Resistance to MBCs is due to the replacement of amino acid

glutamic acid by various amino acids at position 198 (e.g. E198A) of β -tubulin, which is essential for fungal cell replication, rendering the MBC fungicides ineffective. *Z. tritici* populations are still showing resistance to MBC fungicides even though MBC applications to wheat have significantly decreased over the past 20 years (O'Sullivan, 2009).

Demethylation inhibitors (DMIs) were made commercially available by the early 1970s. These fungicides are broad spectrum and are mostly systemic. The main chemical group within the DMI class are the azoles, which are largely represented by the triazoles and also the imidazoles.

Resistance to azoles has increased slowly since the mid-90s; It has been reported that this is attributed to three mechanisms of azole resistance: (i) mutation of the MgCYP51 gene encoding alterations in the target site 14 α -demethylase (Nicolaisen *et al.*, 2009), (ii) over expression of the MgCYP51 gene; (iii) increased efflux of the fungicide, mediated by the overexpression of membrane bound transporters (Cools & Fraaije, 2013). MgCYP51 mutations that can increase resistance to the azole fungicides such as the S524T mutation (Cools *et al.*, 2001) and the V136A + I381V combination (Stammler *et al.*, 2008) have been identified.

The DMIs were the main class of chemicals used to control STB until the introduction of the Quinone outside inhibitors (Qol's). Strobilurins then became the main group of fungicides in the Qol class for the control of cereal disease after their release in the 1990s (Bartlett *et al.*, 2002). Qol's specifically target fungal respiration and provide a broad spectrum of disease control. However, due to their specificity, the risk of fungal pathogens developing a resistance was high and within 2 years of their release to the market, the first reports of the resistance to powdery mildew pathogen *Blumeria graminis f.sp. tritici* started to emerge, (Robinson *et al.*, 2002). By 2002, strains of *Z. tritici* populations with qualitative resistance to Qol's were found in the UK and Ireland (Fraaije *et al.*, 2003). Since 2002, resistance in the *Z. tritici* populations has increased rapidly and now shows complete resistance to Qol's. Molecular analysis of the mitochondrial gene coding cytochrome *b* in resistant isolates of *Z. tritici* has revealed the substitution at amino acid position 143 of glycine by alanine.

Qol's target fungal respiration and this mutation allows the fungus to continue to respire even in the presence of high levels of the fungicide (Kildea *et al.*, 2010).

Succinate dehydrogenase inhibitors (SDHIs) like the azoles are single site inhibitors. The SDHI fungicides interact directly with three (SdhB, SdhC and SdhD) of the four (SdhA, SdhB, SdhC and SdhD) subunits which combine to make the SDHI target site, succinate dehydrogenase. These fungicides inhibit the SDH enzyme, which then disrupts the tricarboxylic acid (TCA) cycle, which is a key process in aerobic respiration (Scalliet *et al.*, 2012). Multisite inhibitors, SDHIs and the sterol 14 α -demethylation inhibitors (DMIs) are still effective for STB control. However, the development of insensitivity in *Z. tritici* to these fungicides has been detected in both Ireland and the UK and poses a threat in the future, to controlling STB in Europe (Dooley *et al.*, 2015; Cools & Fraaije, 2013). Many fungicides can control disease after a leaf becomes infected but only for approximately half of the latent period. For example *Z. tritici* may have a latent period of 14 days in a specific wheat cultivar, but if a fungicide is applied after the leaf has been infected, it may only provide eradicated control for approximately 7 days. Also infection may be so far into the latent period that no quantity of fungicide will control the fungus (HGCA, 2015).

1.5 Aims and Objectives of this MSc

Zymoseptoria tritici grows inside host tissues for typically 10-14 days before the onset of visible disease symptoms (Orton *et al.*, 2011). Reports suggest that little fungal biomass accumulates during this latent period (Dean, 2012); with large increases in fungal growth visible as the fungus shifts into the necrotrophic stage (Hammond-Kosack & Rudd, 2008). Critically, the length of the LP is an important epidemiological factor in subsequent pathogen spread and the generation of epidemics.

The primary objective of this research was to investigate the distribution of latent periods within current commercial cultivars and breeding lines in a natural field environment, with the goal of identifying long LP and short LP varieties as research tools for an in depth analysis of the genetic response of the host during the LP. To commence this initiative, a glasshouse trial was carried out to identify the distribution of LPs within 28 existing wheat cultivars under controlled glasshouse conditions.

The second stage of the research, involved testing cultivars of interest (Stigg, Dunmore, Oakley, JB Diego, Croft, Kielder and Gator) in Norwich, Carlow (Oak Park) and Waterford, in respect to natural field environments of low, medium and high disease pressure.

Finally, the third stage of this research involved investigating the rate of fungal growth in selected cultivars during the *Z. tritici* latent period, by conducting a time-course trial in a glasshouse under controlled conditions (temp set at 18°C) with winter wheat cvs. Stigg (partially resistant) and cv. Gallant (susceptible). This allowed for the amount of fungal biomass present in the leaf tissue to be quantified at set time points (Every 24 hours from 0 to 17 days after inoculation (dai) with the *Z. tritici* Dutch reference isolate IPO323, and also time-points 20, 24 and 28 dai were included).

This research differs from previous work done on the LP of *Z. tritici* in winter wheat due to the natural field environments of high, medium and low disease pressure used over the course of the study and also the comparison of LP from the glasshouse trials.

Previous work on the LP includes Armour *et al*, 2004 where winter wheat cv. Consort was artificially inoculated in a field environment, Viljanen-Rollinson *et*

al, 2005 also artificially inoculated 10 winter wheat cultivars in a field environment.

My hypothesis is that the distribution of LP's identified in the initial controlled glasshouse conditions trial, will follow a similar trend in the natural field conditions trial, and that knowing the LP of *Z. tritici* in different winter wheat cultivars may be useful in the prediction of STB events.

Chapter 2: Investigations into the latent period of *Zymoseptoria tritici* in twenty eight winter wheat cultivars in a controlled environment

2.1 Introduction

Zymoseptoria tritici is an ascomycete fungus which causes Septoria Tritici Blotch (STB) in wheat. *Z. tritici* infects through the stomata and starts its life cycle as a biotroph, growing asymptotically inside the host tissue for 10-14 days before rapidly switching to a necrotroph immediately prior to symptom expression (Keon *et al*, 2007) as described in detail in Chapter 1. While the speed of visible symptom development is linked to temperature and relative humidity, the length of the latent period (Dean, 2012) is an important epidemiological factor in subsequent pathogen spread and the generation of epidemics. Previous reports have highlighted the significant variation that exists in latent periods between different winter wheat cultivars exposed to the same inoculum; cv. Domino suggests an LP of 22.7 days and cv. Regency displayed an LP of 31.7 days (Viljanen-Rollinson *et al.*, 2005).

A glasshouse experiment was designed to establish a dataset of varying LP's of *Z. tritici* from which winter wheat cultivars could be chosen to progress to the field evaluations, where the selected cvs would be exposed to the natural *Z. tritici* inoculum present in the environment at the chosen field sites. This work differs from previous work published on the LP of *Z. tritici* as the host plants were naturally infected rather than artificially infected in the field environment.

2.2 Materials and methods

2.2.1 Artificial inoculation using a mixed isolate inoculum

Twenty eight winter wheat cultivars in the seedling stage (Table 2.1) were used to study the variation of the *Zymoseptoria tritici* latent period. The 2nd leaf of 2-3 week old seedlings was artificially inoculated with a mixed inoculum (8 isolates taken from 2012 Irish field crops, supplied by Steven Kildea, Teagasc (Isolate reference numbers: 172, 307, 325, 347, 396, 409, 505 and 544). Each isolate was grown on potato dextrose agar (PDA) for 4 – 5 days at 18°C, sub-cultured onto fresh PDA and re-incubated at 18°C for a further 4-5 days. The mycelium was then aseptically transferred into 100ml of distilled water and the pycnidiospores suspension read on the haemocytometer. Each isolate suspension was then adjusted to a concentration of 1×10^7 using a template in Microsoft Excel designed by Steven Kildea, Teagasc, Oak Park before being mixed in a 5 litre beaker, containing 2-3 drops of Tween 20 per 2 litres of inoculum). The seedlings were inoculated using a standard household spray bottle and all leaves were sprayed until run off. The inoculated seedlings were then covered for 4 days with plastic bags to mimic ideal conditions for infection to enter the leaf through the stomata. Plastic bags were then removed and plants were kept under glasshouse conditions, at 18°C. This protocol was adapted from the one used by Kema *et al*, 1996. The leaves (except the 2nd leaf) of the seedling plants were kept trimmed in order to facilitate light penetration to the 2nd leaf and disease assessments. Trimming the leaves also inhibits early senescence of the inoculated leaf (Kema *et al*, 1996a).

Table 2.1: Initial 28 winter wheat cultivars screened in glasshouse trials

	Breeder	Winter wheat cultivar
1	Senova, UK	JB Diego
2	Limagrain, UK	Lion
3	Limagrain, UK	Einstein
4	Limagrain, UK	Dunmore
5	Limagrain, UK	Alchemy
6	Limagrain, UK	Claire
7	Limagrain, UK	Stigg
8	Limagrain, UK	Invicta
9	Limagrain, UK	Avatar
10	Limagrain, UK	Horatio
11	Limagrain, UK	Revelation
12	KWS, Germany	Cordiale
13	KWS, Germany	Oakley
14	KWS, Germany	Gator
15	KWS, Germany	Santiago
16	KWS, Germany	Solo
17	KWS, Germany	Grafton
18	KWS, Germany	Croft
19	KWS, Germany	Rowan
20	KWS, Germany	Kielder
21	KWS, Germany	W196 (Dali)
22	KWS, Germany	Lancaster (MH 09-27)
23	KWS, Germany	Twister (MH 10-33)
24	RAGT, UK	Tuxedo
25	RAGT, UK	Warrior
26	RAGT, UK	RW41057
27	RAGT, UK	RW41097
28	RAGT, UK	RW41079

2.2.2 Disease assessments

Zymoseptoria tritici disease presence was visually assessed daily from 0 days after inoculation (Dai) through to 30 Dai by recording the first appearance of pycnidia (FP) on the leaf surface. The seedling experiment was conducted using a randomised block design with five replicates of each cultivar. The experiment was completed twice using the same winter wheat cultivars and *Z. tritici* isolates.

2.2.3 Data analysis

The combined mean data on FP from the two experiments were then subjected to one way ANOVA (in block + model), the 5 reps from experiment 1 were blocks 1-5 and the 5 reps from experiment 2 were blocks 6-10, treatments were compared using Fisher's protected LSD in the statistical software program Genstat 14th Edition (VSN International Ltd, United Kingdom). Results were tabulated and graphed using Microsoft Excel 2010 (Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-7329, USA).

2.2.4 Results

Data analysis indicated a wide diversity of *Z. tritici* LP's with significant difference between winter wheat varieties ($P < 0.001$). The shortest LP observed was 16.7 Dai in cv. Kielder, followed closely by 16.8 Dai in cv. Gator. The longest LP observed was 24.2 Dai in cv. Stigg, followed by cv. Dunmore (21.2 Dai) and cv. JB Diego (21.1 Dai) (Figure 2.2).

Visual assessments showed variance in varietal performance between both replicates of the experiment. In experiment one cvs Lancaster and Alchemy had poor establishment rates under the glasshouse conditions and cvs Warrior, Lion, Revelation and W196 showed healthy leaves with no visible symptoms of *Z. tritici* or other disease throughout the course of the experiment. In experiment two cv. Einstein had a poor establishment rate and cvs Santiago, Grafton, Rowan, RW41057 and RW41079 were highly susceptible to powdery mildew and could not be assessed with confidence for *Z. tritici*.

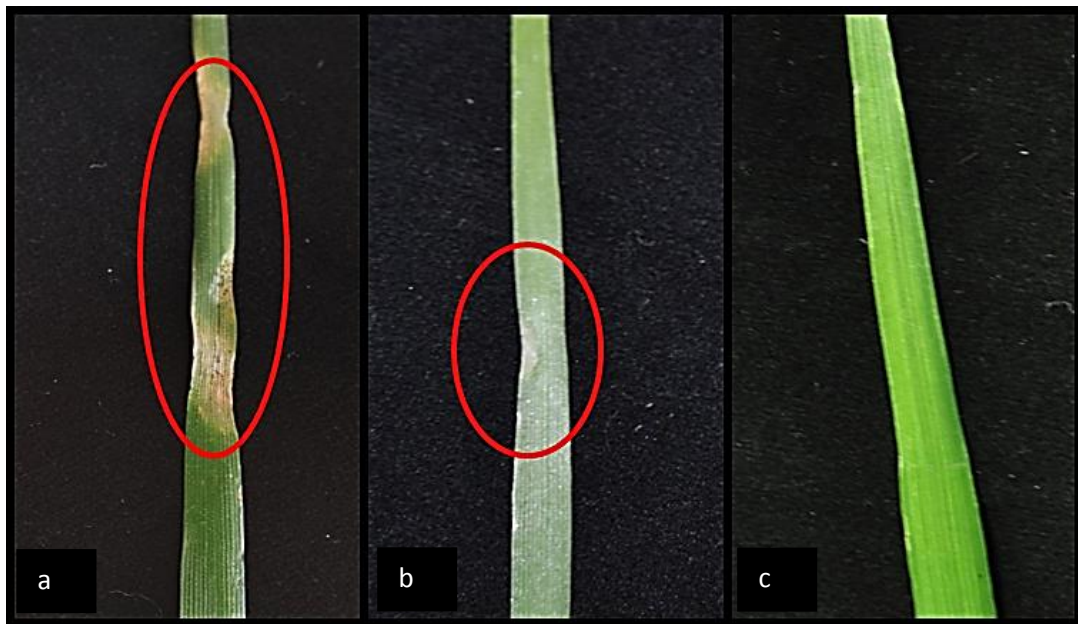


Figure 2.1: Leaves from glasshouse experiment one, displaying visual symptoms of *Z. tritici* disease (a) pycnidia present on cv. Solo, (b) early signs of necrosis on cv. Santiago and (c) no sign of disease on cv. Stigg. All photographs were taken 26 days after inoculation.

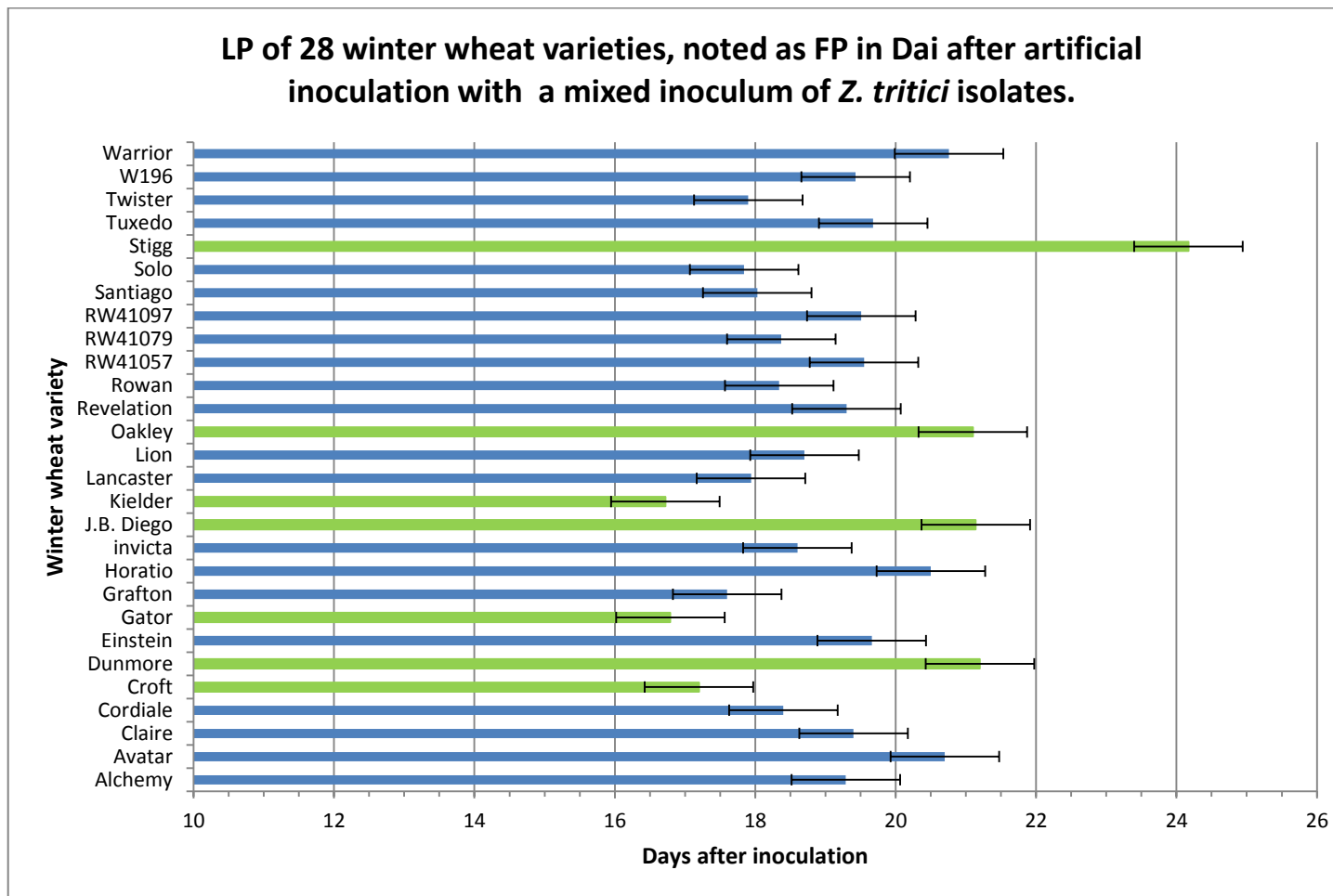


Figure 2.2: Combined means of LP from the two glasshouse experiments on 28 winter wheat cultivars. Blue represents all cvs included in the experiments, green represents the 7 cvs chosen to progress to the field experiments. Glasshouse temperature was set at 18°C and standard error bars: +/- 0.773

2.3 Discussion

The glasshouse experiments were conducted to investigate the distribution of latent periods within current commercial cultivars and breeding lines, with the goal of identifying long LP and short LP cultivars. By initially evaluating 28 cultivars, it enabled a select few to be chosen to progress into the field experiments for further evaluation in the different inoculum pressure environments (low, medium and high).

Seven winter wheat cultivars were chosen from the resulting dataset based on their observed length of LP in days after inoculation which represented partial resistance (long LP) and susceptibility (short LP) to *Z. tritici* under the controlled glasshouse conditions. The seed quality and the establishment rate of the various cultivars was also included in the selection criteria. The selected cultivars are highlighted in green on the graph in Figure 2.2 (Stigg, Dunmore, JB Diego, Oakley, Croft, Gator and Kielder).

Powdery mildew was present in the 2nd glasshouse experiment and although it did not impede the assessment of STB on the majority of the 28 cultivars, its presence did eliminate some varieties from the assessments.

Cultivars which were showing no visible symptoms of disease in experiment 1 were included in experiment 2 and showed susceptibility to *Z. tritici*, therefore it may be possible that the Inoculum in the 1st experiment did not penetrate the leaf surfaces during the initial post inoculum incubation period in plastic bags. The cultivars chosen for further evaluation in the field experiments recorded consistent disease symptoms in experiments 1 and 2.

The poor establishment rate of some cultivars may have been due to poor seed quality or the seed been in a dormancy period, therefore a test for germination rate and also pre-incubation of the seeds prior to sowing should be conducted in future glasshouse experiments.

Chapter 3: Investigations into the latent period of *Zymoseptoria tritici* in seven winter wheat cultivars in a field environment

3.1 Introduction

As severe epidemics of STB can reduce winter wheat yields by up to 50% (Fones & Gurr, 2015; O'Driscoll *et al.*, 2014), intensive chemical control measures are required to facilitate good yields. Due to increasing resistance in European populations of *Z. tritici* to the Quinone outside Inhibitors (QoI) and Methyl Benzimidazole Carbamate (MBC) classes of fungicides (Cools & Fraaije, 2013; Kildea *et al.*, 2010) along with azoles (Dooley *et al.*, 2015) and more recently Succinate Dehydrogenase Inhibitors (SDHI's) (Sierotzki, 2013), alternative STB controls need to be investigated such as breeding for high yielding resistant winter wheat cultivars (cvs).

The latent period (Dean, 2012) of *Z. tritici* is an important phase during the pathogen's life cycle, and represents when the pathogen gains entry to the host's leaves through the stomata through to the formation of pycnidia. The mechanisms which affect the rate of fungal growth within the leaf tissues are not fully understood, and the growth rate varies between winter wheat cultivars. Consequently, the genetic responses to the pathogen-host interaction during this asymptomatic LP remain under-investigated. Previous research has suggested that the LP can vary between 6 and 36 days depending on the combination between wheat cultivar and fungal isolate (Lee *et al.*, 2013). The duration of the LP is directly affected by environmental conditions such as temperature, humidity and inoculum pressure (Viljanen-Rollinson *et al.*, 2005). By investigating the approximate LP length of winter wheat cultivars under different environmental conditions, I hypothesise, the data collected will follow a similar trend in regards to LP length as in the glasshouse trials and knowing the LP of *Z. tritici* in winter wheat may further assist in the prediction of STB events and could be useful in the interpretation of timing fungicide applications.

The objective of these experiments was to test this hypothesis by taking into account three different disease pressure sites and seven winter wheat cultivars with varying partial resistance / susceptibility to *Z. tritici*.

3.2 Materials and methods

Seven winter wheat cultivars (Table 3.1) were chosen from the initial glasshouse trials based on the variation of their LP's (longer / shorter) to be screened in field trials over 3 consecutive years from 2013 – 2016. Cv. Oakley was replaced by cv. Gallant after the first year of field trials due to severe infection of yellow rust, and therefore no measurement data are available for cv. Oakley in 2014.

Table 3.1: Winter wheat varieties screened in field trials over 3 consecutive growing seasons.

Variety	Latent Period (days) from glasshouse trials	STB resistance score (from breeders variety data sheet)
Stigg	24.17	8 (Limagrain, UK)
Dunmore	21.20	6 (Limagrain, UK)
JB Diego	21.14	5 (Senova, UK)
Croft	17.20	5 (KWS, Germany)
Kielder	16.72	5 (KWS, Germany)
Gator	16.79	5 (KWS, Germany)
Oakley	21.10	6 (KWS, Germany)
Gallant*	12.00*	5* (Syngenta, Switzerland)

*Cultivar Gallant replaced cv. Oakley in the 2015 and 2016 field trials.

Field trials were conducted during the seasons of 2013-14, 2014-15 and 2015-16 at three locations Oak Park (52.861°N, 6.915°W), Waterford (52.297°N, 7.111°W) in Ireland and Norwich (52.622°N, 1.221°E) in the UK. These locations were chosen in respect to previous field evaluations that indicated they typically represented environments of low (Norwich), medium (Oak Park) and high (Waterford) disease pressure. All trials were laid out in a complete randomised block design with 5 replicate blocks with 7 winter wheat varieties included in each block, but the Norwich trial unfortunately lost 3 of the varieties in 2014 to very poor plant establishment (cvs Kielder and Gator) and also infection of yellow rust (cv. Oakley) (Figure 3.1).

The Waterford trial showed a lower establishment rate in cv. JB Diego (Figure 3.2) in comparison to the other 6 cvs. The Oak Park trial had good establishment rates in 2013-2014 but unfortunately as in Norwich cv. Oakley was severely infected by yellow rust (Figure 3.3).

The experimental design was completed using the online tool Edgar II (Experimental Design Generator And Randomiser, by James K.M. Brown, Cereals Research Department, John Innes Centre, Norwich, England). Norwich plots were 1.5m x 6.0m, Oak Park plots were 2.5m x 6.0m and Waterford plots were 1.5m x 3.0m in size. Seed rates were determined based on environmental conditions and sowing date (Table 3.2). Plant establishment rates were recorded at GS 23 in Waterford and GS 24 in Oak Park during the 2013-14 and 2014-15 seasons, GS 24 in Waterford and GS 25 in Oak Park during the 2015-16 season. The Oak Park trial displayed low establishment rates during the 2014-15 and 2015-16 growing seasons due to flooding of the site during the winter months.



Figure 3.1: Norwich trial site 2013-2014, poor establishment of cvs Kielder and Gator.



Figure 3.2: Waterford trial site 2013-2014, poor establishment of cv. JB Diego



Figure 3.3: Oak Park trial site 2013-2014, yellow rust on cv. Oakley

Table 3.2: Seed rates and plant establishment data for Waterford, Oak Park and Norwich across all three growing seasons.

		Waterford	Oak Park	Norwich
2013-2014	Seed/m ²	260.0	260.0	260.0
	Mean plant establishment /m ²	153.1	138.8	74.0
2014-2015	Seed/m ²	369.0	369.0	260.0
	Mean plant establishment /m ²	148.3	96.5	*
2015-2016	Seed/m ²	400.0	360.0	260.0
	Mean plant establishment /m ²	276.9	90.9	*

*Data unavailable

Infection of *Zymoseptoria tritici* was allowed to develop naturally in each trial by applying fungicide products to control yellow rust and mildew but that had minimal effect on *Z. tritici*. Agronomic data recorded during all three growing seasons is presented in Table 3.3. All other crop management was conducted to a commercial standard, aimed at minimising crop stress. Due to outbreaks of powdery mildew and yellow rust at all three locations each year, the trials received an application of Corbel® (fenpropimorph and cyclohexanone), BASF and Amistar® (azoxystrobin), Syngenta (0.5/0.5 half rate) using a knapsack sprayer with compressed air. These fungicides were used due to their lack of effect on *Z. tritici* (Dooley *et al.*, 2015).

Table 3.3: Agronomic details of field trials in Oak Park, Waterford and Norwich including sowing date, number of cultivars (cvs) included, fungicide details and growth stage applied and respective products and rates as well as date of first disease assessment and respective harvest date.

Field Trial	Location	Sowing date	Number cvs	Fungicide detail		First disease assessment	Harvest date
				GS applied	Products and rates		
Year 1	Oak Park	15.10.13	6	<i>GS60 – June 13th</i>	<i>Amistar at 0.5 l/ha / Corbel at 0.5 l/ha</i>	15.05.14	05.09.14
Year 1	Waterford	14.10.13	6	<i>GS60 – June 12th</i>	<i>Amistar at 0.5 l/ha / Corbel at 0.5 l/ha</i>	14.05.14	13.08.14
Year 1	Norwich	23.09.13	4	<i>GS32</i>	<i>Amistar at 1.0 l/ha / Talius at 0.25 l/ha</i>	15.05.14	07.08.14
				<i>GS59</i>	<i>Amistar at 1.0 l/ha / Talius at 0.25 l/ha</i>		
Year 2	Oak Park	24.10.14	7	<i>GS39 – May 26th</i>	<i>Amistar at 0.5 l/ha / Corbel at 0.5 l/ha</i>	29.04.15	08.09.15
Year 2	Waterford	24.10.14	7	<i>GS39 - May 25th</i>	<i>Amistar at 0.5 l/ha / Corbel at 0.5 l/ha</i>	28.04.15	25.08.15
Year 2	Norwich	23.09.14	7	<i>GS32 - no date available</i>	<i>Amistar at 1.0 l/ha / Talius at 0.25 l/ha</i>	29.04.15	10.09.15
				<i>GS59 - no date available</i>	<i>Amistar at 1.0 l/ha / Talius at 0.25 l/ha</i>		
Year 3	Oak Park	13.10.15	7	<i>GS45 – May 2nd</i>	<i>Amistar at 0.5 l/ha / Corbel at 0.5 l/ha</i>	17.05.16	16.08.16
Year 3	Waterford	13.10.15	7	<i>GS45 – May 3rd</i>	<i>Amistar at 0.5 l/ha / Corbel at 0.5 l/ha</i>	16.05.16	12.08.16
Year 3	Norwich	23.09.16	7	<i>GS32 - no date available</i>	<i>Amistar at 1.0 l/ha / Talius at 0.25 l/ha</i>	12.07.16	23.08.16
				<i>GS59- no date available</i>	<i>Amistar at 1.0 l/ha / Talius at 0.25 l/ha</i>		

3.3 Weather, disease and latent period assessments

3.3.1 Weather data

Daily weather data (Table 3.4 and Table 3.5) for the Irish trial sites was collected at the Oak Park Automatic Weather Station (AWS) situated on the grounds of Teagasc, Oak Park, Carlow (52.861°N, 6.915°W) and the Johnstown AWS (52.174°N, 6.293°W), closest weather station to the Waterford trial site (43km), situated on the grounds of Teagasc, Johnstown Castle, Wexford by Met Eireann (www.met.ie). The mean daily temperature and total monthly rainfall was calculated for May, June and July for 2014, 2015 and 2016. Humidity data was available for the Oak Park site but was not available for Waterford and Norwich sites.

Weather data (Table 3.6) for the Norwich trial site at Morley was collected at the Lowestoft weather station (approx. 46km from trial site), Monckton Avenue, Lowestoft (52.286°N, 1.433°E), by the Met Office UK (www.metoffice.gov.uk).

Table 3.4: Waterford maximum, minimum and mean temperature (°C) and total monthly rainfall (mm).

Year	2014			2015			2016		
Month	May	June	July	May	June	July	May	June	July
Mean Temp (°C)	11.4	14.0	16.7	10.3	13.1	14.0	11.4	14.5	15.2
Max Temp (°C)	18.0	22.9	23.3	18.9	22.1	20.0	18.8	20.3	22.4
Min Temp (°C)	4.9	6.8	9.5	4.6	5.3	6.1	4.4	7.7	8.6
Total Rainfall (mm)	63.6	35.2	35.2	145.0	35.0	116.0	70.3	93.5	44.1

Table 3.5: Oak Park maximum, minimum and mean temperature (°C), total monthly rainfall (mm) and humidity (%).

Year	2014			2015			2016		
Month	May	June	July	May	June	July	May	June	July
Mean Temp (°C)	11.9	14.5	16.9	10.2	13.4	14.6	12.3	15.1	16.0
Max Temp (°C)	20.9	24.9	27.6	19.2	24.0	23.4	21.9	23.7	27.5
Min Temp (°C)	5.2	5.3	7.2	1.6	3.6	6.4	3.4	4.7	6.8
Total Rainfall (mm)	78.6	61.9	24.6	89.4	29.7	79.4	61.6	61.7	29.6
Mean Humidity (%)	83.8	80.1	77.5	79.0	74.9	80.2	80.6	83.7	83.3

Table 3.6: Norwich maximum, minimum and mean temperature (°C) and total monthly rainfall (mm). Estimated by Met Office UK.

Year	2014			2015			2016		
Month	May	June	July	May	June	July	May	June	July
Mean Temp (°C)	13.2	15.2	18.4	11.6	14.5	17.7	12.6	15.2	18.3
Max Temp (°C)	16.7	19.0	21.7	15.7	18.7	22.0	16.0	18.4	22.4
Min Temp (°C)	9.7	11.3	15.1	7.5	10.2	13.4	9.1	11.9	14.1
Total Rainfall (mm)	94.8	28.5	59.3	47.0	25.4	152.6	55.0	102.4	38.3

3.3.2 Disease assessment

Each plot in Oak Park and Waterford contained 20 tagged plants (Figure 3.4) for repeat *Z. tritici* disease assessments in all of the 3 years field trials. Each plot in Norwich contained 4 tagged plants in the 2013-14 field trial, this number was increased to 10 tagged plants in the 2014-15 and 2015-16 field trials.

Symptoms of STB were assessed on the 2nd last emerged leaf (referred to below as “2nd leaf”) and flag leaf (last emerged leaf) of each tagged plant every three/four days during early May to late July. The percentage of leaf area containing STB was visually assessed and only lesions bearing visible pycnidia were recorded. The disease assessment software Distrain (Tomerlin & Howell, 1988) was used prior to assessment to provide training and familiarity on disease scoring.

Due to scheduling restrictions during the 2015-2016 growing season, assessments for disease progression were carried out on the flag leaf only of the 20 tagged plants per plot at the Waterford and Oak Park trial sites.

Plots were harvested in each year using a adapted combine harvester (Sampo 2010, Sampo Rosenlew Ltd, Finland) in Norwich and Oak Park; and by hand harvesting in Waterford (a 1m² quadrat was hand harvested and mechanically threshed using a Wintersteiger, Austria LD180 Laboratory thresher). The total grain from each plot was weighed and the moisture content determined by drying in a laboratory oven at 70°C for 48 hours. Yields were then calculated as t/ha at 15% moisture content.

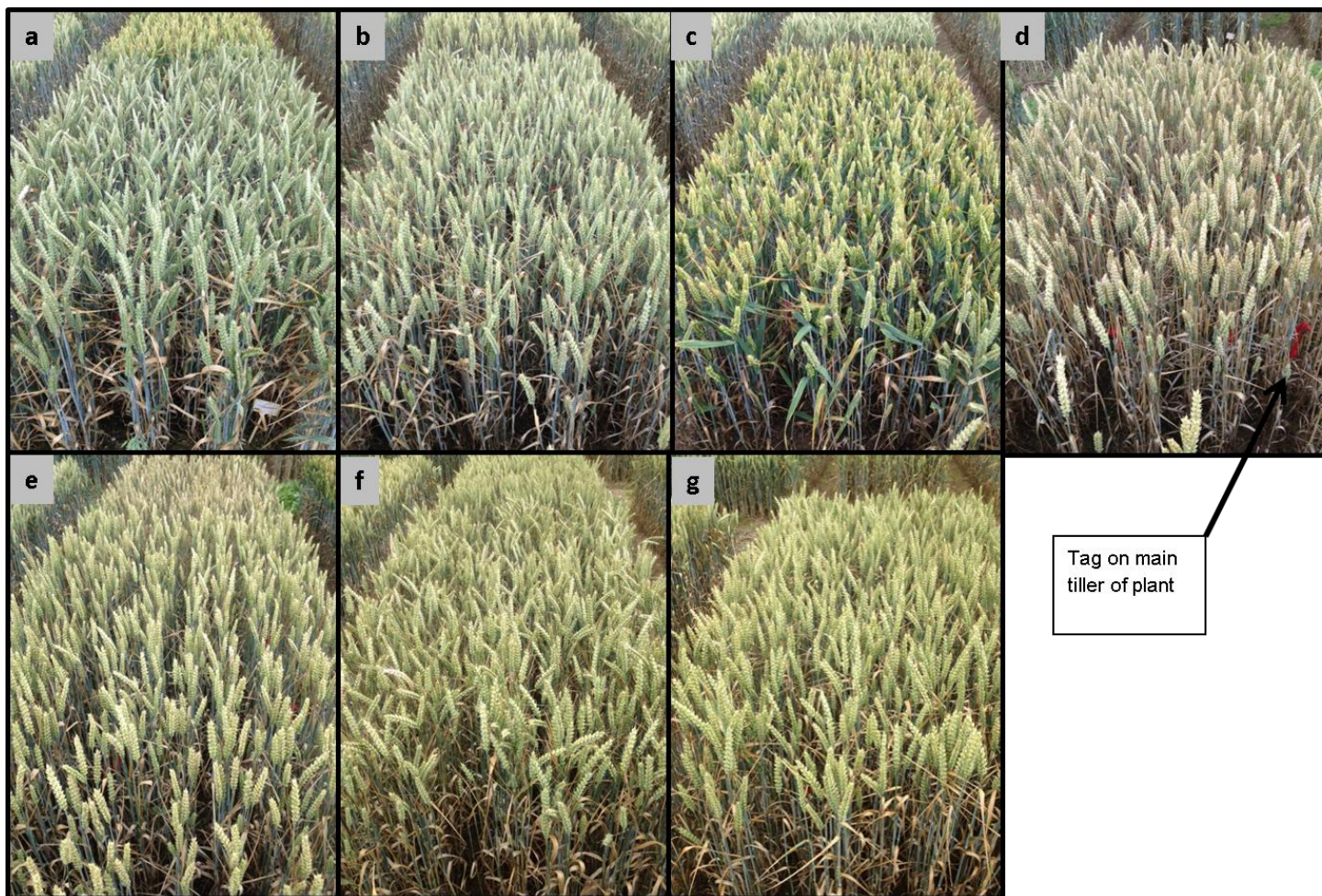


Figure 3.4: Tagged plants in Waterford on 13.07.2016. (a) cv. Stigg, (b) cv. Dunmore, (c) cv. JB Diego, (d) cv. Croft, (e) cv. Kielder, and (f) cv. Gator and (g) cv. Oakley

3.3.3 Latent period of *Zymoseptoria tritici* in winter wheat

To study the variance in latent periods across cultivars, flag leaf and 2nd leaf emergence dates were recorded in Oak Park and Waterford during the 2014-2015 growing season in an effort to achieve an estimated LP of *Z. tritici*. For this it was assumed that infection did not occur until the 2nd and flag leaves had started to emerge, at which stage they would potentially be exposed to *Z. tritici* inoculum. To achieve this, the date when greater than 50% of the 20 tagged plants per plot were bearing pycnidia were recorded to establish an approximate LP end point.

3.4 Data analysis

Data recorded in the field was collected using a Motorola MC75A6 data logger (% *Z. tritici* disease progression and leaf emergence dates) from all three growing seasons and entered into a pre-formatted excel sheet. Means of the % disease progression at each sampling date on both the 2nd leaf and flag leaf of the 20 tagged plants were calculated for each varietal replicate across all three growing seasons and sites. These means, along with LP and yield data were then subjected to one way ANOVA (in randomised blocks) and treatments were compared using Fisher's protected LSD in the statistical software program Genstat 14th Edition (VSN International Ltd, United Kingdom). Results were tabulated and graphed using Microsoft Excel 2010 (Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-7329, USA).

3.5 Results

It was observed from the data collected from the flag leaves across all trial sites over the three consecutive years that cv. Stigg had the longest LP. However, the rate of disease progression on cv. Stigg was slower during its extended LP and then increased rapidly once visual symptoms of *Z. tritici* infection appeared. Even so, the amount of disease on cv. Stigg was limited because the rapid phase of disease progress did not necessarily finish before the leaves became senescent. The shortest LP was observed on cv. Gator in 2014 and cv. Gallant in 2015 and 2016. Cultivars Croft, Dunmore, JB Diego and Kielder's LP's were between those and cv. Stigg.

3.5.1 Progression of STB disease at high inoculum pressure site - Waterford, Ireland.

During the 2013-2014 growing season winter wheat cv. Oakley suffered severe infection from yellow rust across all three trial locations and had to be eliminated from the field trial. The analysed STB disease progression data from the remaining six cultivars (Stigg, Dunmore, JB Diego, Croft, Kielder and Gator) in Waterford showed high significant difference ($P < 0.001$) between cultivars throughout all assessment dates in 2014. Winter wheat cv. Gator, scoring a 5 on the KWS data sheet for STB resistance displayed a consistently high level of disease on both 2nd leaf and flag leaf, winter wheat cv. Stigg which scores an 8 on the Limagrain data sheet for STB resistance recorded consistently low disease progression on both the 2nd leaf and flag leaf. The remaining four cultivars (Dunmore, JB Diego, Croft and Kielder) displayed moderate levels of resistance.

This is confirmed below on the graph in Figure 3.5, where cv. Gator appears to be the most susceptible of the six cultivars to *Z. tritici* with first symptoms of disease appearing on the 2nd leaf by the 23rd May +/- 2 days. Disease then progressed to 30% within 12 subsequent days (5th June) and reached 80% within a further 11 days (16th June). In contrast, cv. Stigg displayed first symptoms of disease on the 2nd leaf on the 31st May +/- 2 days, 8 days after cv. Gator. Disease then progressed at a slower rate, reaching 30% within 17 days

(16th June, on the same date cv. Gator was at 80% disease severity). Cultivar Stigg did not progress to 80% for a further 23 days (10th July), confirming its high partial resistance to *Z. tritici* infection. Cultivars Dunmore, JB Diego, Croft and Kielder all displayed a similar rate of disease progression on their 2nd leaves, all reaching 30% disease within 3 days of each other (9th – 12th June) and progressing to 80% disease within 4 days of each other (20th – 24th June).

The rate of disease progression on the respective flag leaves (Figure 3.6) followed a similar trend, with disease progressing quickly on cv. Gator once first signs of infection appeared. For cv. Stigg, disease severity only reached 70% before natural senescence occurred. Cultivars Dunmore, JB Diego and Croft grouped together showing moderate resistance, but cv. Kielder showed earlier infection and increased progression levels unlike on the 2nd leaf.

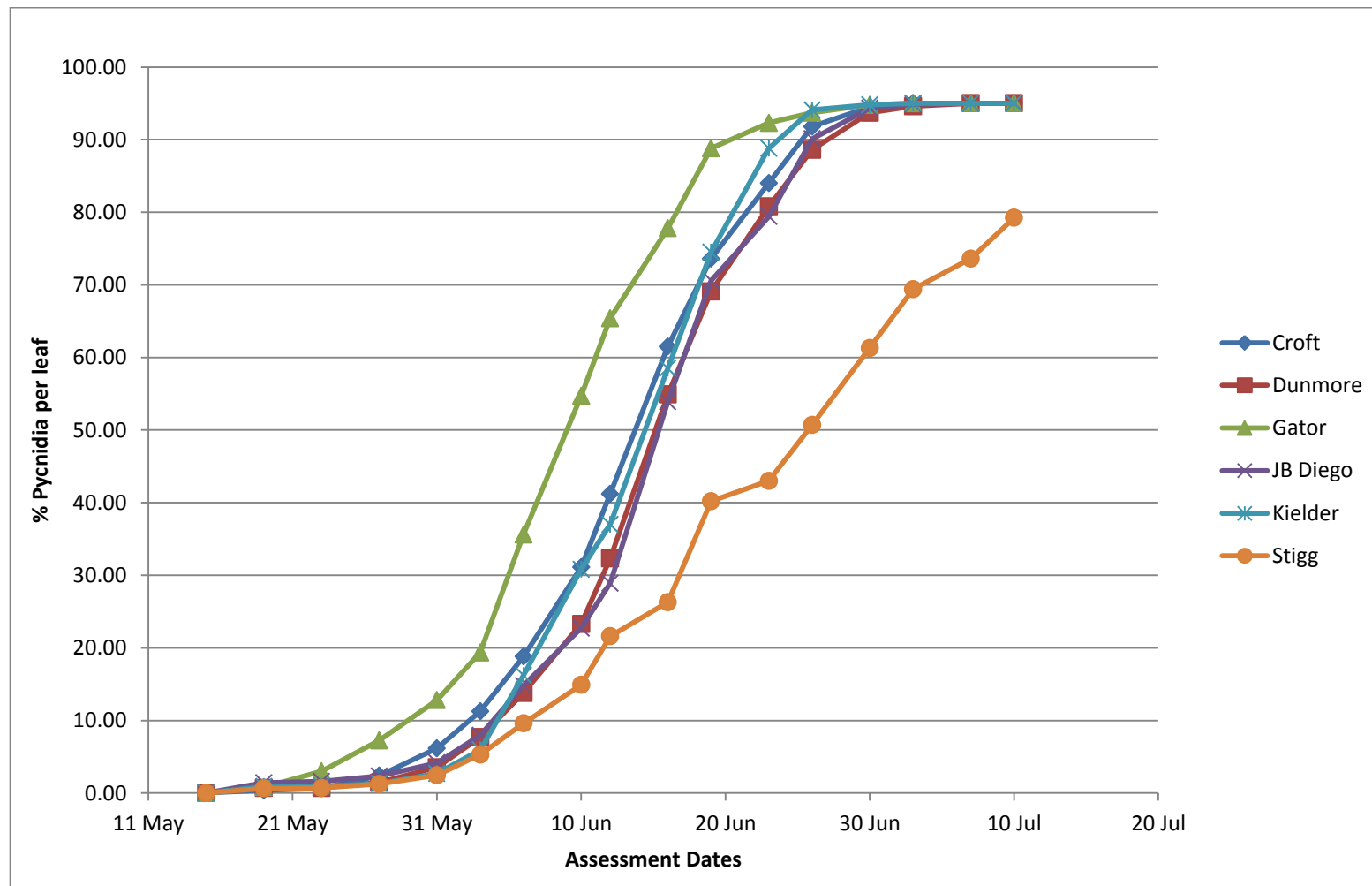


Figure 3.5: Covered by pycnidia as a percentage area of leaf area on the 2nd leaf in Waterford in 2014.

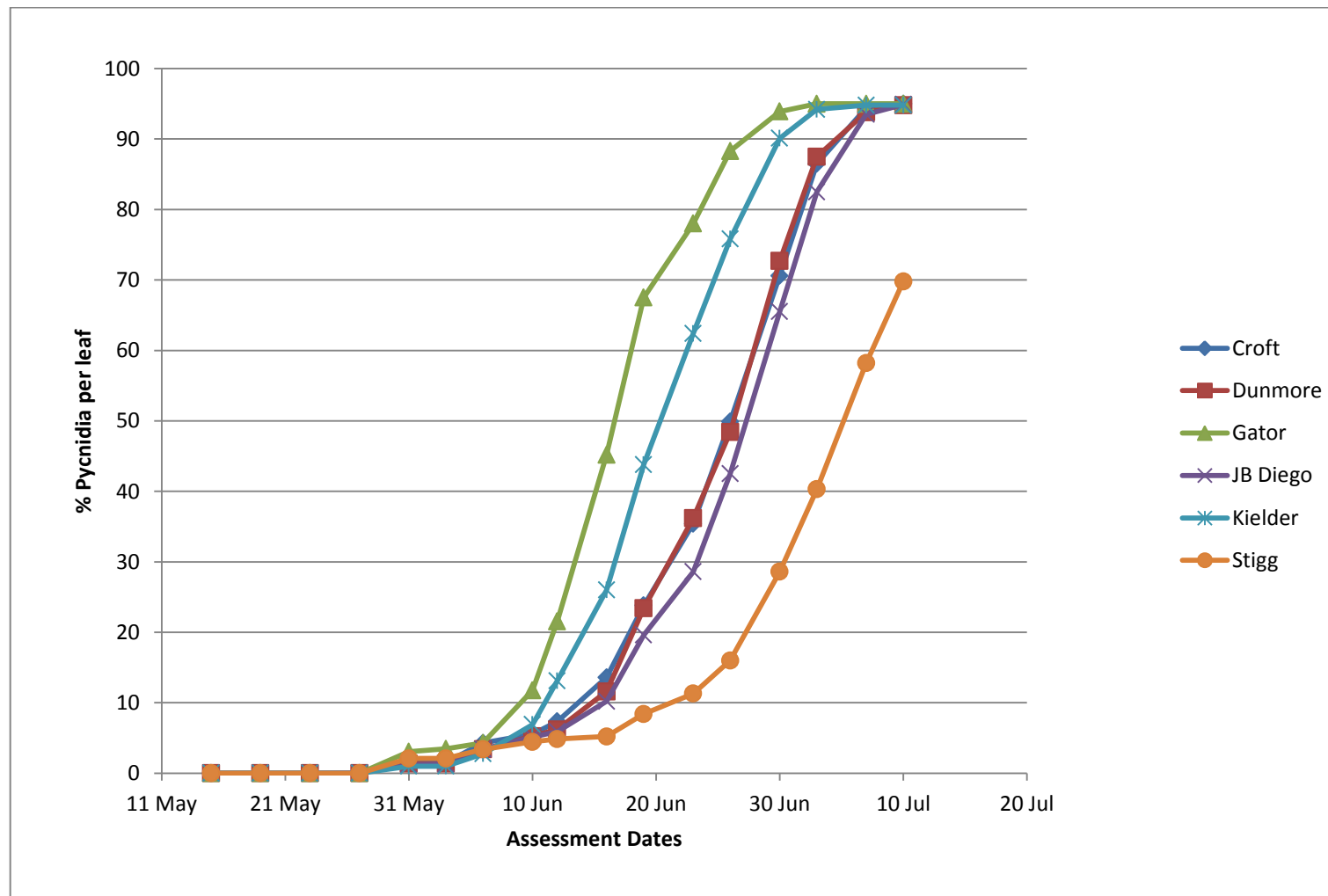


Figure 3.6: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Waterford in 2014.

For the 2nd year of the study at the Waterford site, cv. Gallant (considered highly susceptible to *Z. tritici* infection, scoring a 5 on the Syngenta data sheet) was substituted to replace cv. Oakley due to Oakley's susceptibility to yellow rust. The other change made was that visual assessments were started approximately 3 weeks earlier than the 2014 assessments in an effort to capture leaf emergence dates of the 2nd and flag leaves.

During the 2014-2015 growing season, Figure 3.7 shows 0% disease severity on the 2nd leaf for the first 4 weeks of assessments until approx. 26th May across all seven winter wheat cultivars. After this date the various cultivars showed significant differences ($P < 0.001$) in disease progression. Cultivars Kielder and Gallant's disease severity performed similarly in the first 10 days after symptoms appeared. Kielder's disease severity progressed from approximately 3% on the 31st May (+/- 2 days) to 30% by the 10th June after which the rate of progression decreased slightly and disease severity reached 80% by the 11th July (+/- 2 days). In contrast cv. Gallant did not reach 30% disease until the 16th June, six days after Kielder, at which point disease then progressed at a greater rate to 80% within a further 19 days on the 5th July. Disease progression rates of cvs Gator and Croft were equivalent reaching 30% on approximately 21st June and progressed to 80% within 2 weeks (7th July) and cvs Dunmore and JB Diego also showed similarity reaching 30% on approximately the 4th July and progressing to 80% by the 13th July. In contrast cv. Stigg displayed high partial resistance to disease, with severity progressing gradually up to 30% by approximately 14th July +/- 2 days and reaching 80% eleven days later on the 25th July.

Cultivar Kielder's disease severity had slowed on the flag leaf in comparison with the 2nd leaf (Figure 3.8). Disease progressed slowly to 20% by approx. 6th July and then progressed at a greater rate to 80% within eleven days (17th July). Cultivar Gallant steadily reached 30% disease on the 17th June and progressed to 80% by the 13th July. The other 5 cultivars performed similarly up to 10%, at which stage disease severity on cvs Dunmore, JB Diego, Croft and Gator also progressed at a greater rate up to 80% within approx. ten days. Again cv. Stigg remained the most resistant of the cultivars, with disease severity not reaching 80% until the 4th August at which stage the other cultivars were senescing.

In comparison to the 2013-2014 growing season, disease progression rates followed similar trends with the exception of cv. Kielder which showed increased susceptibility to *Z.tritici* on the 2nd leaf in 2015. Also the first symptoms of disease appeared 2 weeks later in 2015 on the 2nd leaf and flag leaf than in 2014. Cultivar Stigg maintained its high partial resistance; however cvs Kielder (2nd leaf) and Gallant (flag leaf) took over from cv. Gator (both leaves) as appearing to be the most susceptible of the cultivars.

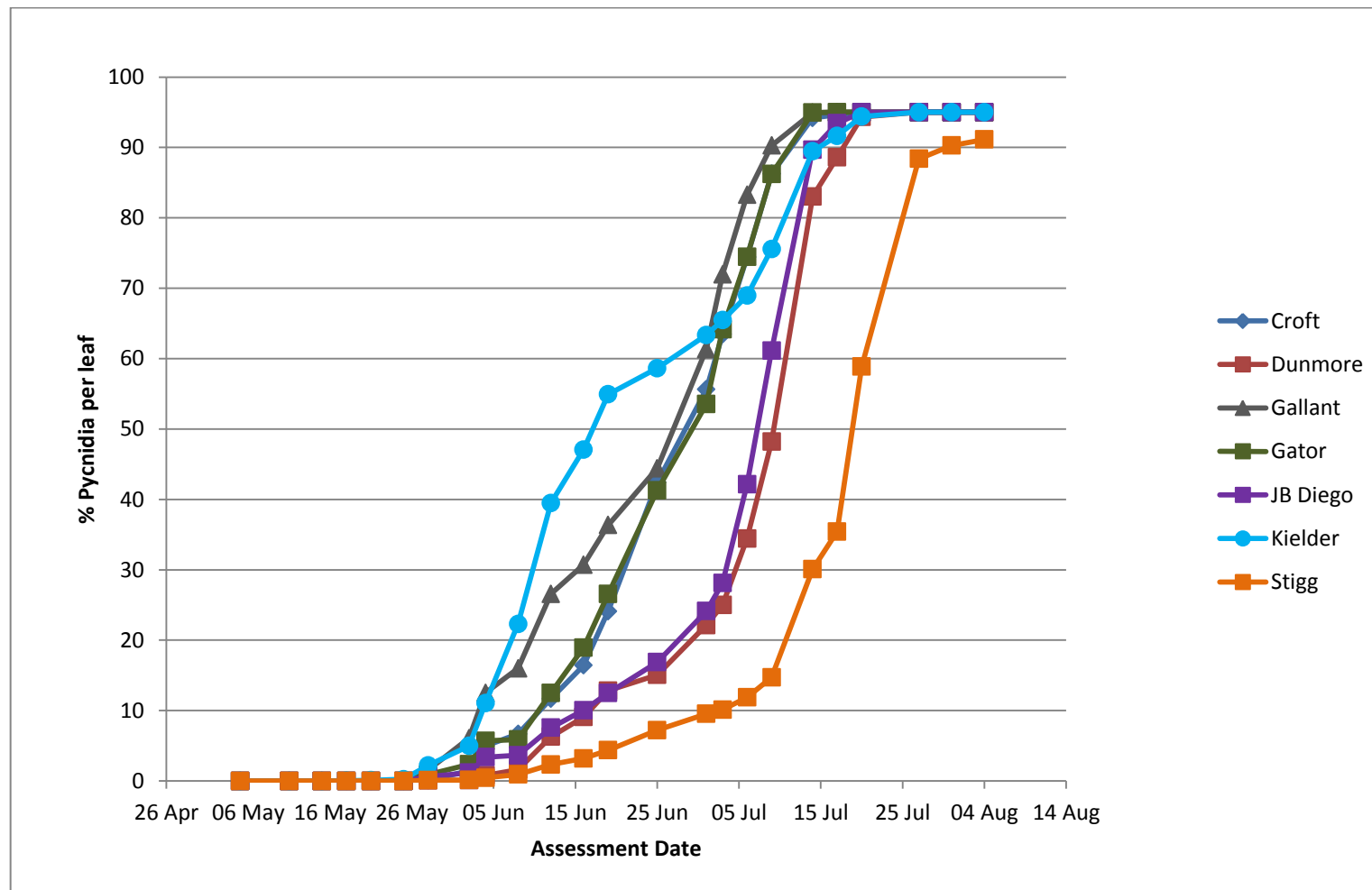


Figure 3.7: Covered by pycnidia as a percentage area of leaf area on the 2nd leaf in Waterford in 2015.

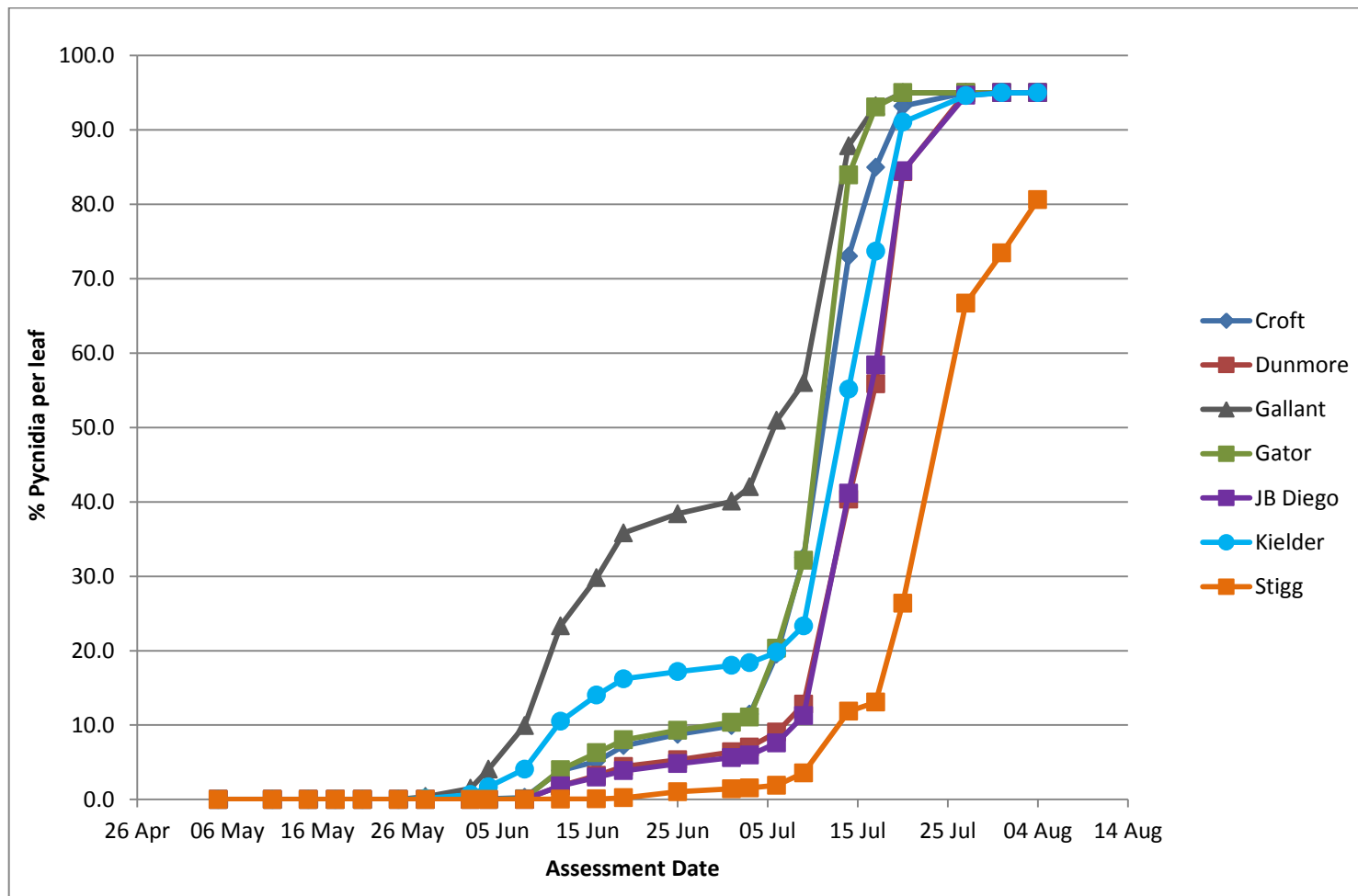


Figure 3.8: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Waterford in 2015.

The flag leaf data during the 2015-2016 growing season showed 0 % disease across all seven cultivars during the first three assessment dates (16th, 19th and 23rd May 2016). Unfortunately, due to technical matters, assessments had to be halted during this critical time of evaluation and could not resume until 1st July 2016, at which stage disease severity had progressed to 90% (cv. Gallant), 60% (cv. Gator), 55% (cv. Kielder), and 45% (cvs Dunmore and JB Diego). Disease severity remained at low levels (15%) up to this date on cv. Stigg and progressed to approx. 45% by the last assessment date of the 2016 season (13th July) (Figure 3.9). Focusing on the last three assessments dates (1st, 6th and 13th July 2016), the rate of disease progression across cultivars followed similar trends to the 2014-2015 and 2013-2014 growing seasons, cv Stigg displayed the highest rate of resistance to *Z.tritici*, reaching approximately 70% by the 13th July in 2014 and only 10% by the same date in 2015. Cultivar Gallant showed high susceptibility to the pathogen in 2015, reaching 50% by the 6th July and 90% by the final assessment date and cv. Gator was the most susceptible in 2014, when its disease severity was at 95% by approx. 1st July. Disease progression rates within each winter wheat cultivar showed high significant differences across all three years. Cultivar Stigg displayed disease severity of 15% (2016), 1% (2015) and 35% on approximately 1st July. Cultivar Kielder displayed disease severity of 55% (2016), 15% (2015) and 90%. The remaining five cultivars followed similar trends across the three years.

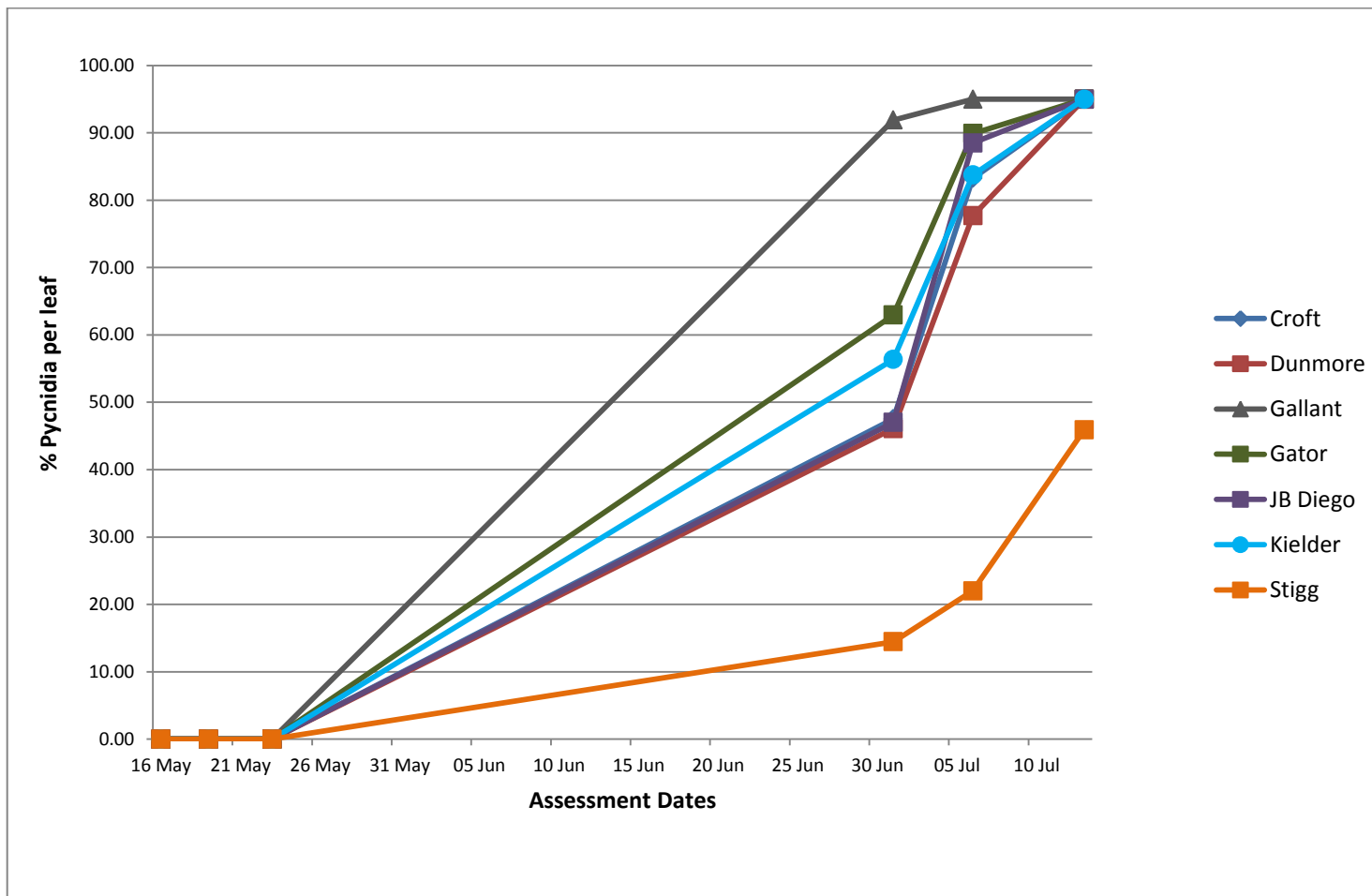


Figure 3.9: Covered by pycnidia as a percentage area of leaf area on the Flag leaf in Waterford in 2016.

3.5.2 Progression of STB disease at medium inoculum pressure site - Oak Park, Carlow, Ireland.

During the 2013-2014 growing season in Oak Park, significant differences ($P < 0.001$) were observed between disease progression on all winter wheat cultivars. Cultivar Gator displayed first symptoms of *Z. tritici* infection on the 2nd leaf by the 26th May (3 days after cv. Gator in Waterford), disease severity reached 30% by the 9th June and progressed to 80% within 11 days by the 20th June (Figure 3.10). Winter wheat cv. Stigg remained the most resistant to infection with 0 % disease on the 2nd leaf until 6th June, this progressed to 30% by the 2nd July and disease severity did not rise above 70% before the last assessment date on the 16th July. All the remaining cultivars displayed moderate severity of disease resistance throughout the assessment period, reaching 30% between the 15th and 18th June and progressing to 80% between the 26th and 29th June.

Disease progression on the flag leaves (Figure 3.11) followed similar trends, with visible disease recorded on cv. Gator by the 30th May +/- 2 days, progressing to 30% by the 24th June and progressing at a greater rate to 80% by the 30th June. Disease symptoms appeared in cv. Stigg started on the 9th June and progressed to less than 50% by the 16th July. The remaining cultivars Dunmore, JB Diego, Croft and Kielder followed a similar trend to both the flag leaf and the Waterford trial by grouping together. Disease severity on all cultivars reached 30% by the end of June and progressed to 80% by mid-July.

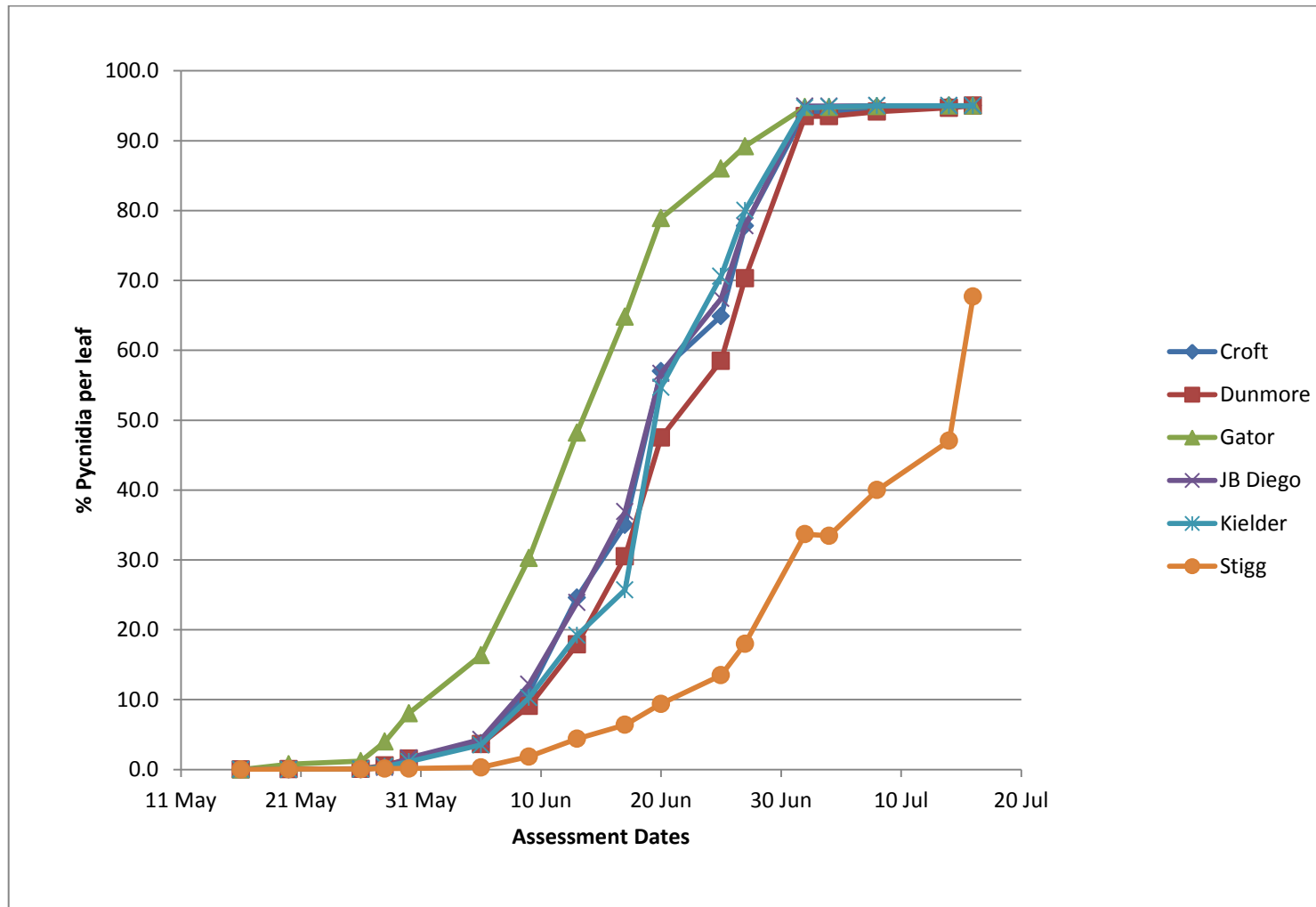


Figure 3.10: Covered by pycnidia as a percentage area of leaf area on the 2nd leaf in Oak Park in 2014.

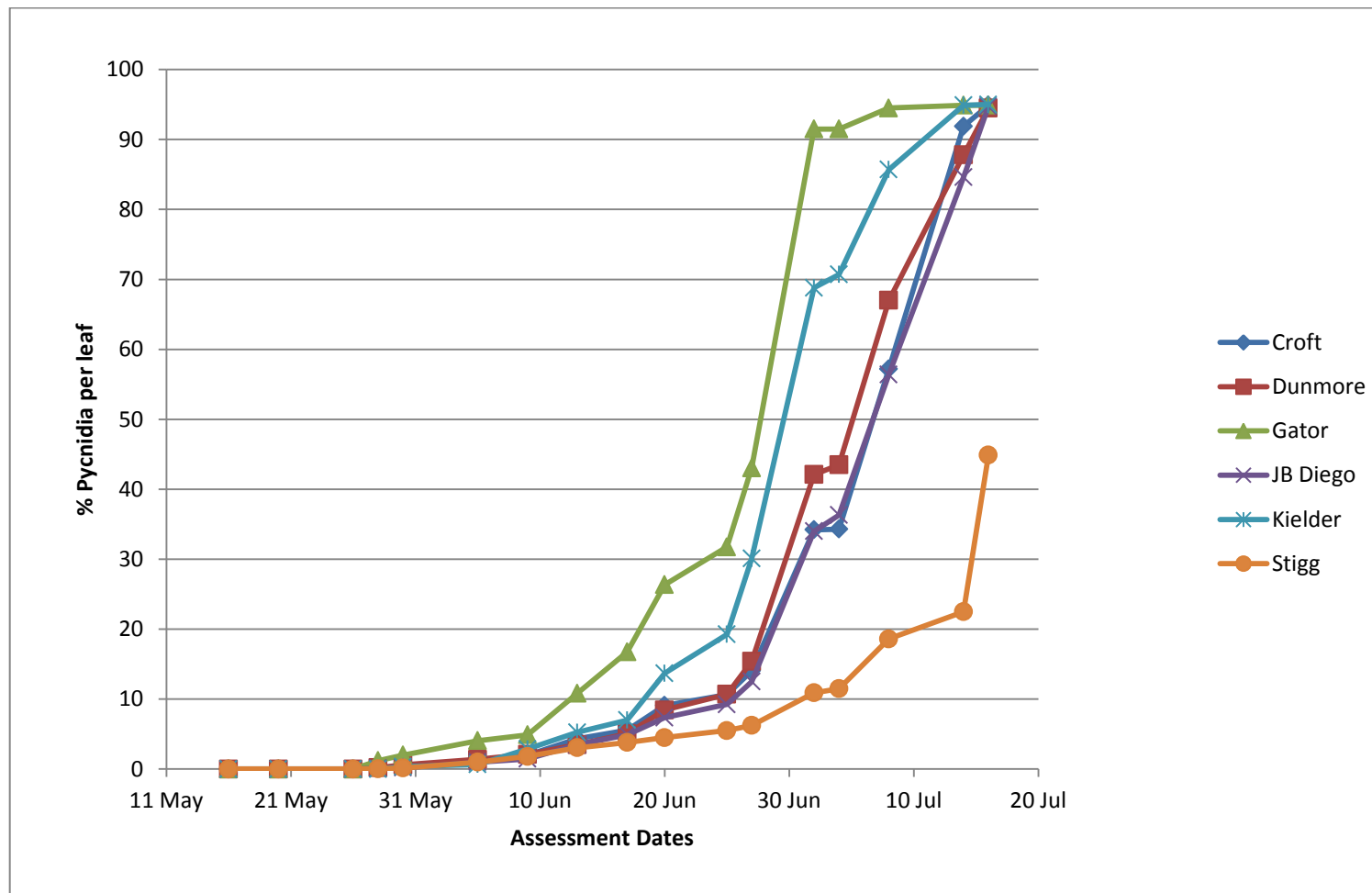


Figure 3.11: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Oak Park in 2014.

During the 2014-2015 growing season all seven cultivars in Oak Park showed low levels of disease on the 2nd leaf until mid-June, when disease severity on cv. Gallant started to rise to 30% by the 27th June, progressing to 80% by the 10th July. Cultivars Dunmore, Croft, Gator, Kielder and JB Diego showed disease severity of 30% between the 1st and 13th July, progressing to 80% within a further 10 days. Cultivar Stigg was still showing relatively low levels of disease up to the 16th July and then progressed at a greater rate to 80% by the 30th July (Figure 3.12).

The flag leaf of all seven cultivars in Oak Park remained clear of disease until the end of June. When disease began to develop in early July (all cultivars except Stigg displayed approx. 10% between the 7th and 16th July and progressed to 80% within 9 days (16th to 25th July). In comparison, Stigg showed 30% disease severity on the 21st July and progressed to less than 55% by the last assessment date of 27th July (Figure 3.13).

To compare the 2014-2015 growing season to the previous year; disease progression across cultivars followed similar trends i.e. Stigg consistently showed the highest resistance to *Z. tritici*, cv. Gallant took over from cv. Gator as the more susceptible cultivar and all other cultivars displayed moderate resistance to the pathogen. The first symptoms of disease appeared 3 weeks later during the 2014-2015 growing season and once infection was present in the leaf the progression rate was faster than during the 2013-2014 growing season.

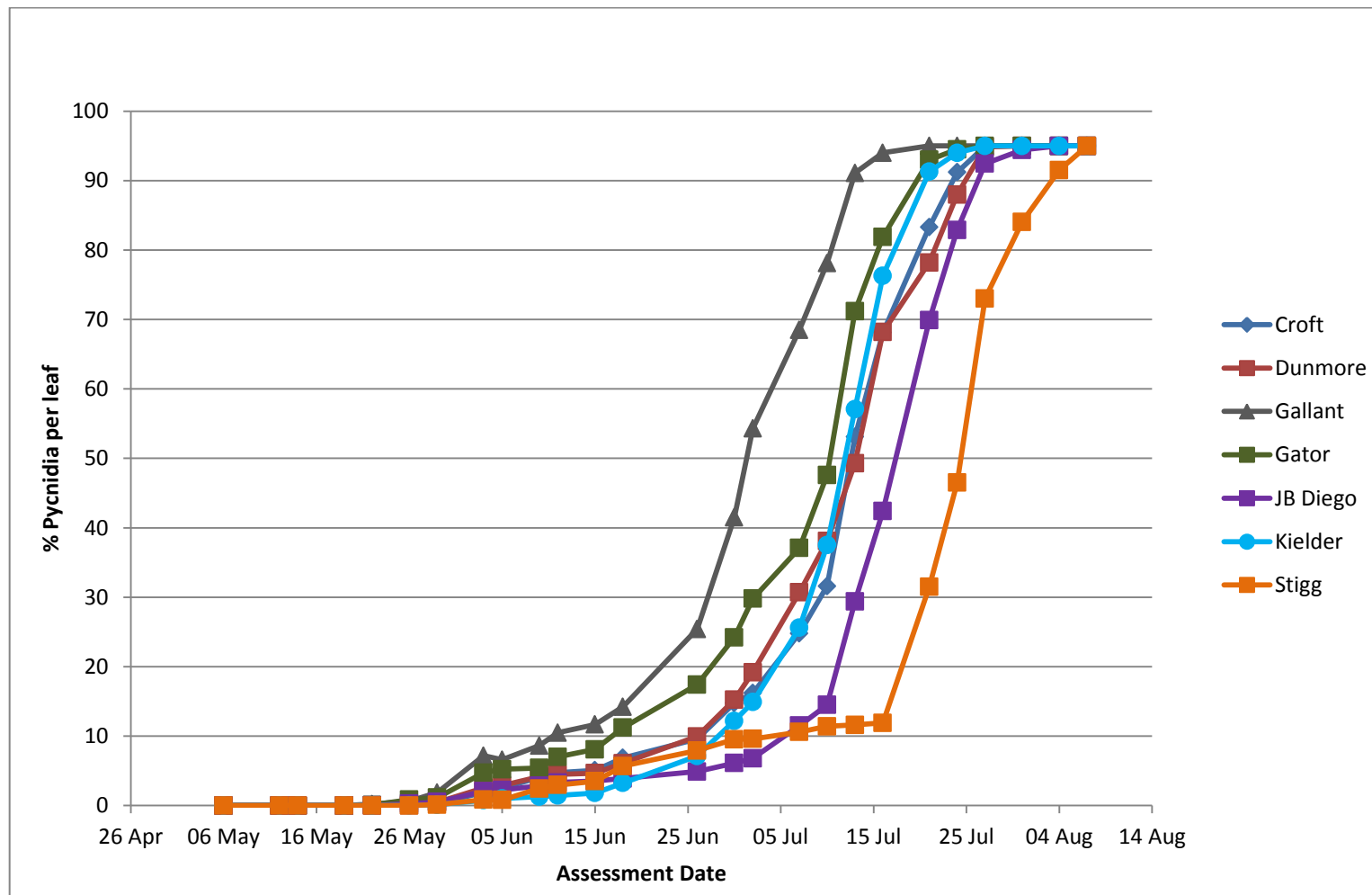


Figure 3.12: Covered by pycnidia as a percentage area of leaf area on the 2nd leaf in Oak Park in 2015.

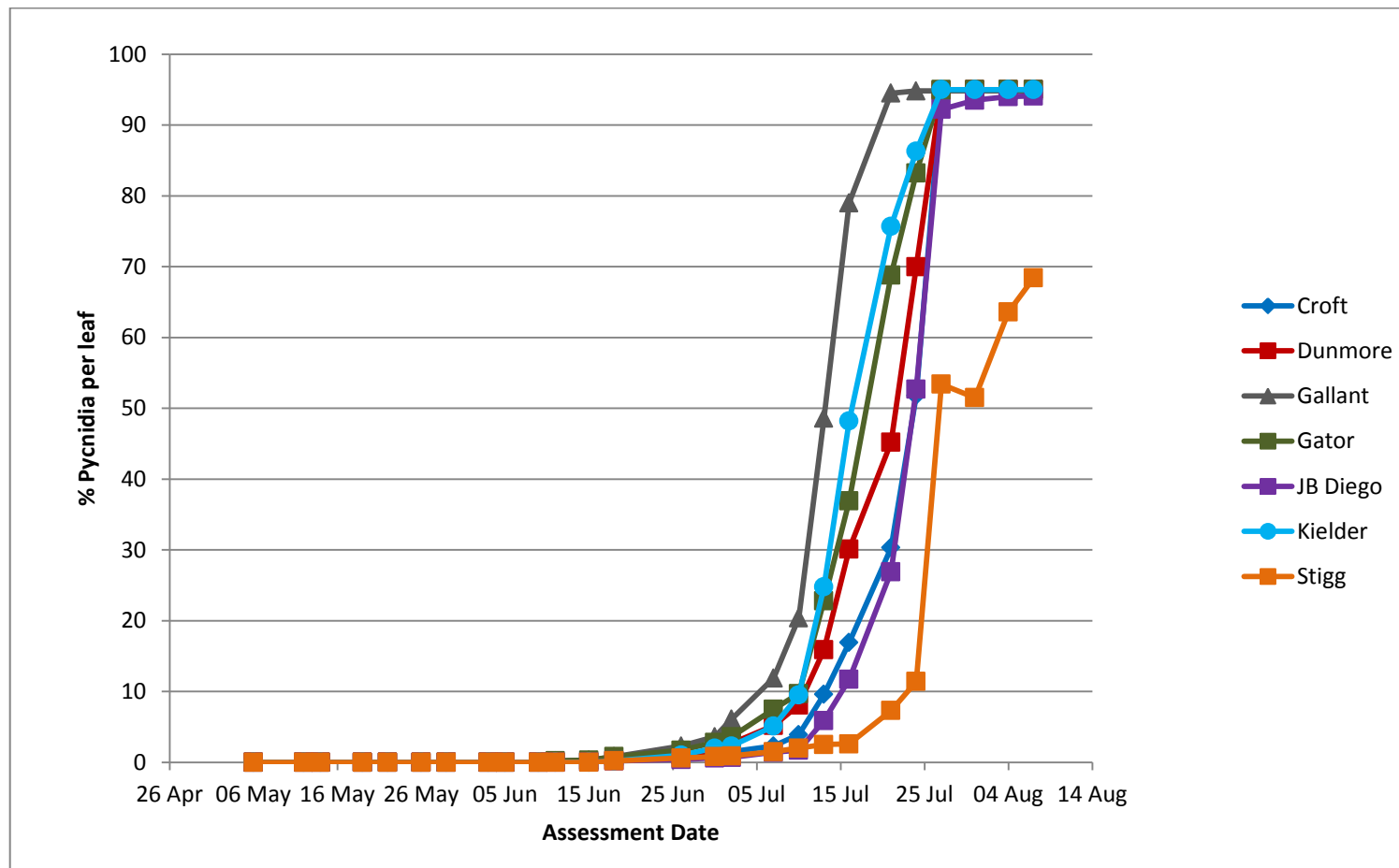


Figure 3.13: Covered by pycnidia as a percentage area of leaf area the flag leaf in Oak Park in 2015.

During the 2015-2016 growing season in Oak Park, assessments were also stopped between the 24th May and the 28th June. Figure 3.14 shows significant differences ($P < 0.001$) between all seven winter wheat cultivars. However, cv. Gallant had advanced to 85% disease by the 28th June and increased by a further 10% within seven days. From the 5th July up to the last assessment date on the 19th July it was seen to flat line at 95% disease. During the same time intervals cvs Kielder, Dunmore and JB Diego had approx. 30%, cvs Gator and Croft had approx. 15% and cv. Stigg had less than 10% disease by the 28th June. Winter wheat cv. Stigg is shown to have increased steadily but slowly up to just below 50% disease by the 19th July, where cvs Dunmore, Croft, JB Diego, Gator and Kielder's progression increased at a faster rate until the 5th July and then tapered off until reaching 95% disease on the 19th July.

Focusing on the last three assessments dates (28th June, 5th and 19th July 2016), the rate of disease progression across varieties followed similar trends to the 2014-2015 and 2013-2014 growing seasons, cv. Stigg displayed the highest rate of resistance to *Z.tritici*, reaching approximately 45% by the 16th July in 2014 and only 10% by 21st July in 2015. Cultivar Gallant showed low disease severity in 2015, reaching only 10% by the 5th July but progressed to 95% within 2 weeks by the final assessment date. Cultivar Gator was the most susceptible in 2014, when its disease severity was at 45% by 27th June. Disease progression rates within each winter wheat cultivar showed no significant differences between 2014 and 2016, but showed significant difference in 2015. Cultivar Stigg displayed disease severity of 5% (2016), 0% (2015) and 5% in the last 3 days of June, whereas cv. Gator displayed disease severity of 15% (2016), 1% (2015) and 45%. The remaining five cvs followed similar trends across the three years.

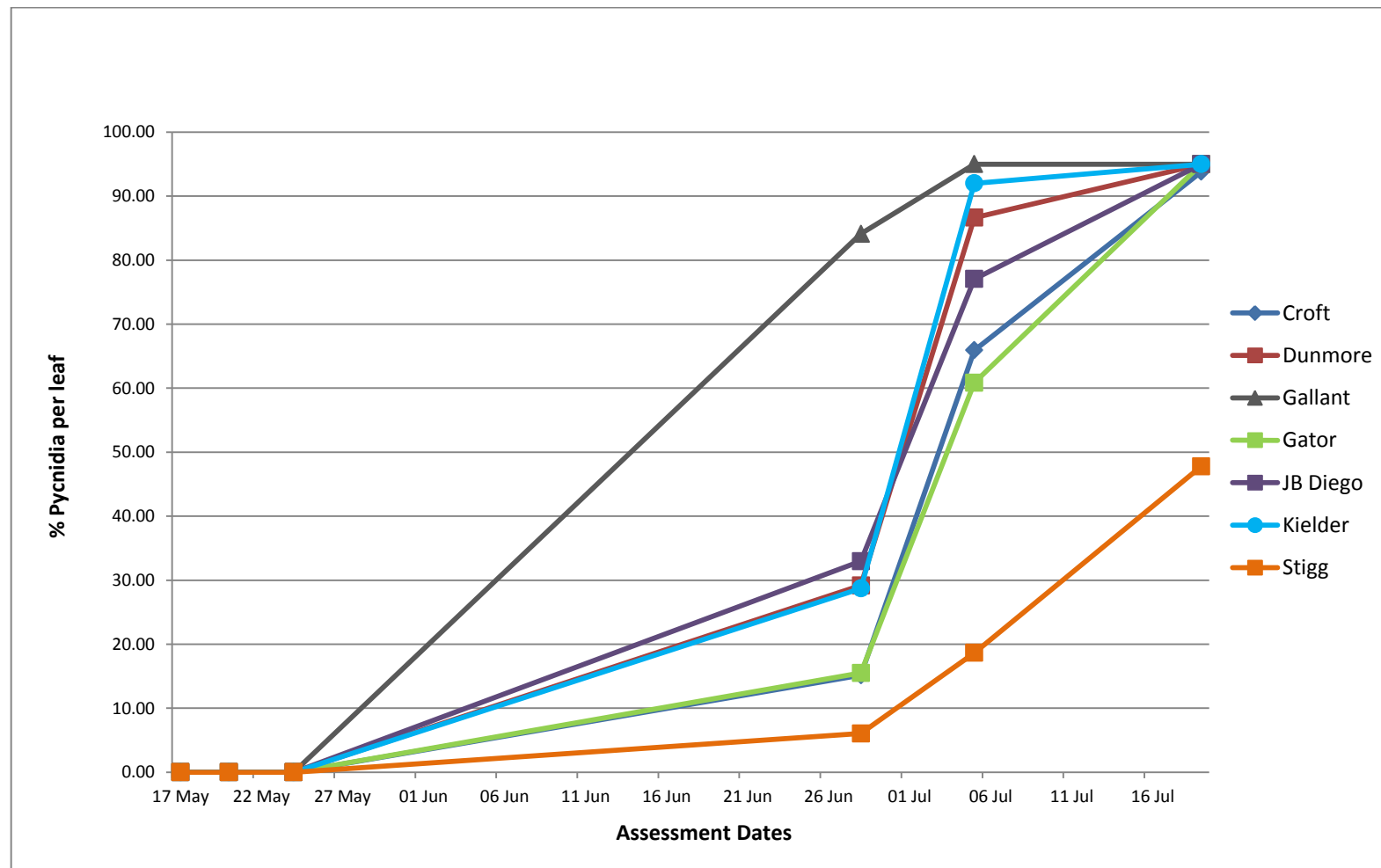


Figure 3.14: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Oak Park in 2016.

3.5.3 Progression of STB disease at low inoculum pressure site - Norwich, United Kingdom.

During the 2013-2014 growing season in Norwich (low disease pressure environment) winter wheat varieties Oakley, Kielder, and Gator had to be excluded from the data analysis due to severe infection from yellow rust, which despite best efforts could not be kept under control by the use of fungicides. Disease assessments commenced on the 13th June on both the 2nd leaf and flag leaf of the four tagged plants per plot. Unfortunately the dates when disease symptoms first appeared were not captured for the Norwich trial as they were for the Waterford and Oak Park trials. However, Figure 3.15 showed clear indication of the progression of disease from the 1st assessment date through to the last assessment date (11th July). Winter wheat cv. Stigg clearly displayed its high partial resistance to *Z. tritici* by very slowly increasing from 5% disease on the 13th June to just under 20% disease on the 11th July. Winter wheat cv. Croft (35% disease on the 13th June and rising to 90% by the 30th June) appeared to be more susceptible than cvs Dunmore and JB Diego (30% disease on the 27th June while cv. Croft had 75% disease on the same date). From the 27th June, cv. Dunmore's disease severity progressed steadily until reaching 95% on the 11th July, cv. JB Diego's disease severity increased at a faster rate to 95% between the 27th June and the 1st July before flat lining.

On the flag leaves, disease appeared at approx. the same date 13th June but at a lower severity (Figure 3.16) and progressed steadily with only cv. Croft reaching 95% by the 11th July. Cv. Stigg's disease severity also remained low on the flag leaf of the plant, only reaching 20% by the end of the assessment period.

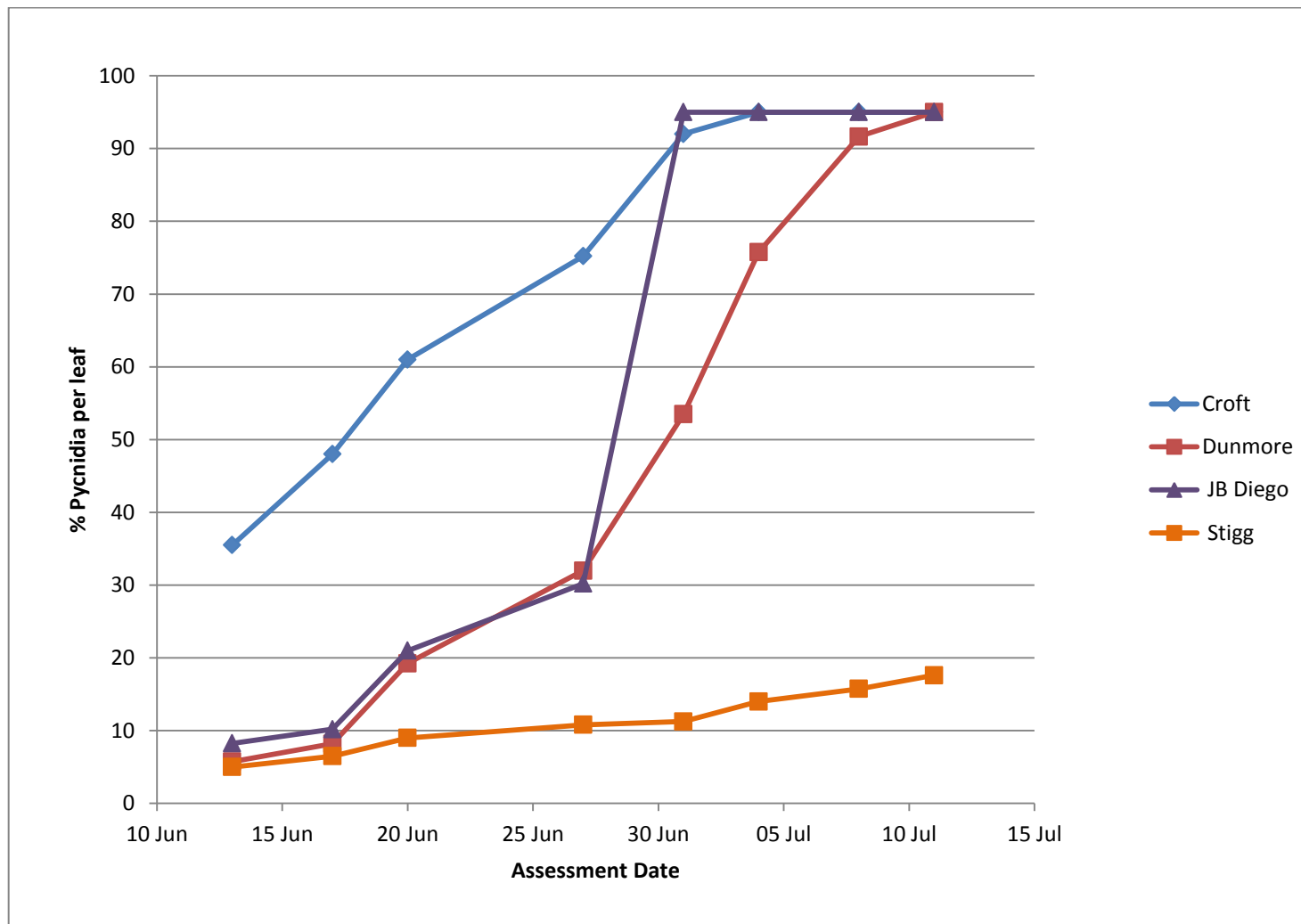


Figure 3.15: Covered by pycnidia as a percentage area of leaf area on the 2nd leaf in Norwich in 2014.

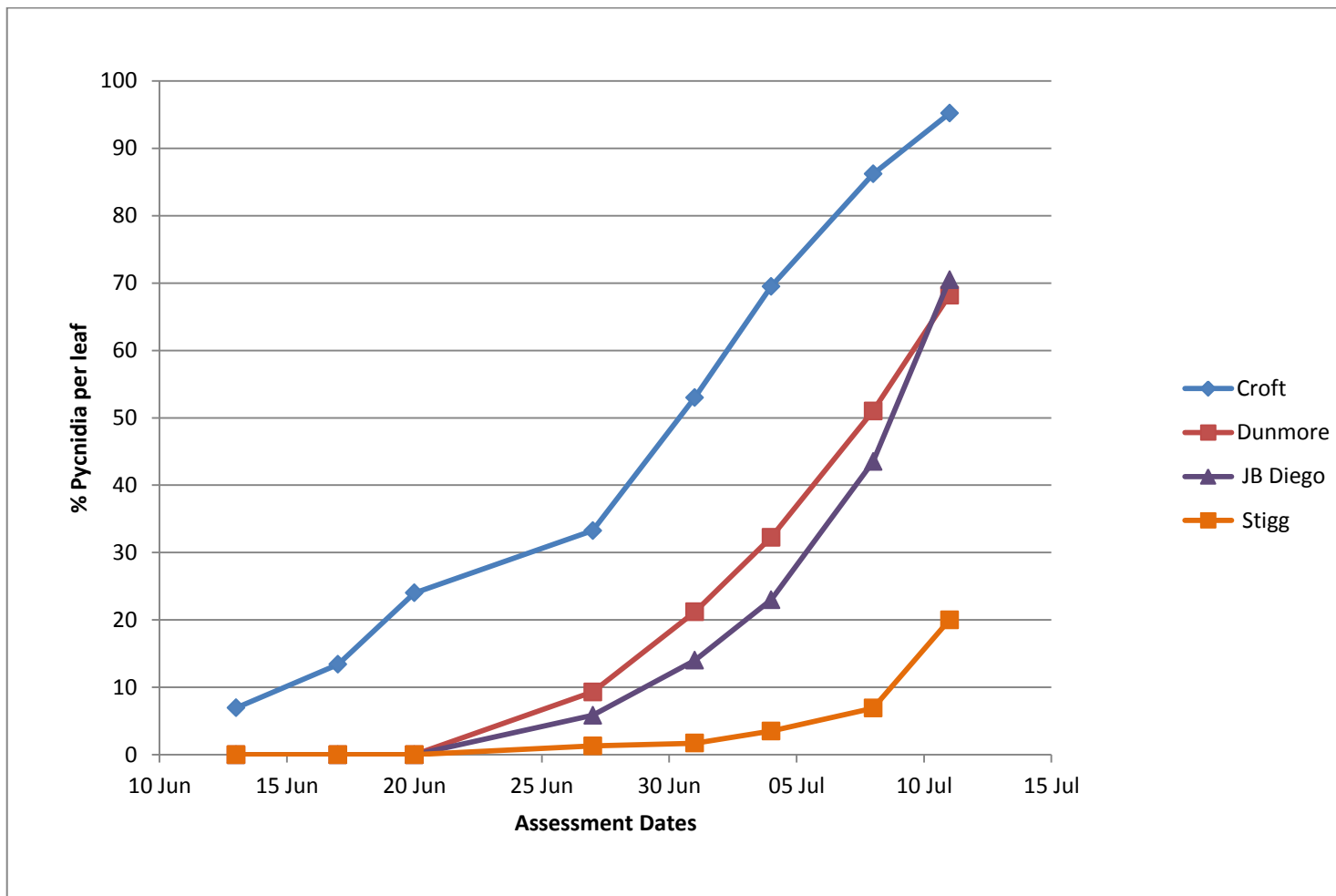


Figure 3.16: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Norwich in 2014.

During the 2014-2015 growing season there were two disease scorers due to the availability of one individual to carry out the assessment throughout the entire growing season, therefore there was fluctuation in the collected data based on the individuals' perception of disease severity. The fluctuation was so slight, it was not necessary to adjust the data before statistical analysis. *Z.tritici* severity was significantly lower than the previous year's on both 2nd and flag leaves with severity progressing to only 40% by the last assessment date on the 14th July (Figure 3.17 and 3.18). However varietal susceptibility / resistance were consistent with the 2013-2014 data for cvs Stigg, Dunmore, JB Diego and Croft. Cultivar Stigg continues to show high partial resistance with disease severity progressing to only 15% on the 2nd leaf and 5% on the flag leaf. Yellow rust disease severity was also lower than the previous year and cvs Kielder and Gator along with cv. Gallant showed *Z.tritici* severity which could be recorded and the data analysed.

In comparison to the Waterford and Oak Park trials cv. Gallant showed higher levels of resistance to *Z.tritici* with cvs Gator, Croft and JB Diego appearing more susceptible to infection of the pathogen.

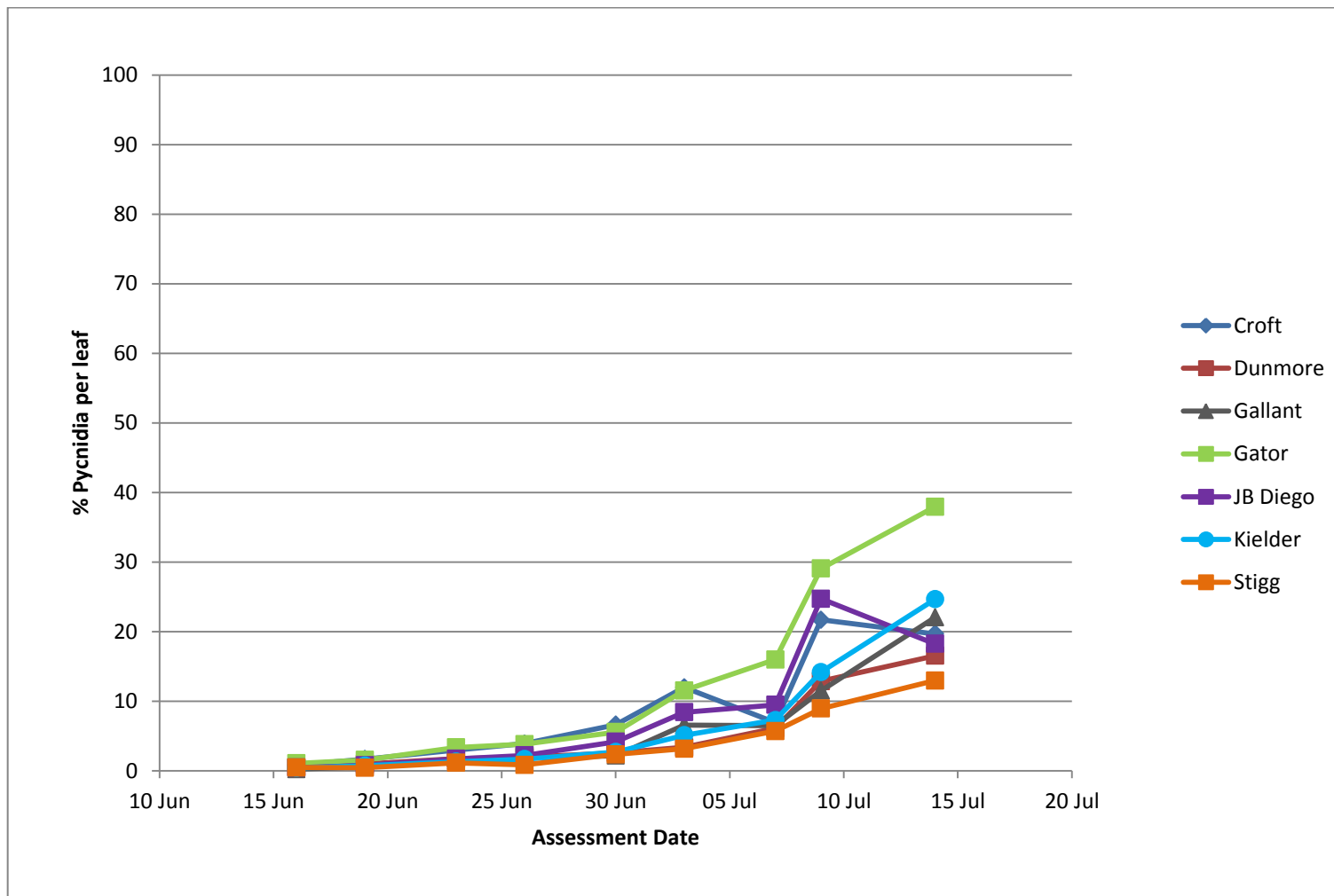


Figure 3.17: Covered by pycnidia as a percentage area of leaf area on the 2nd leaf in Norwich in 2015.

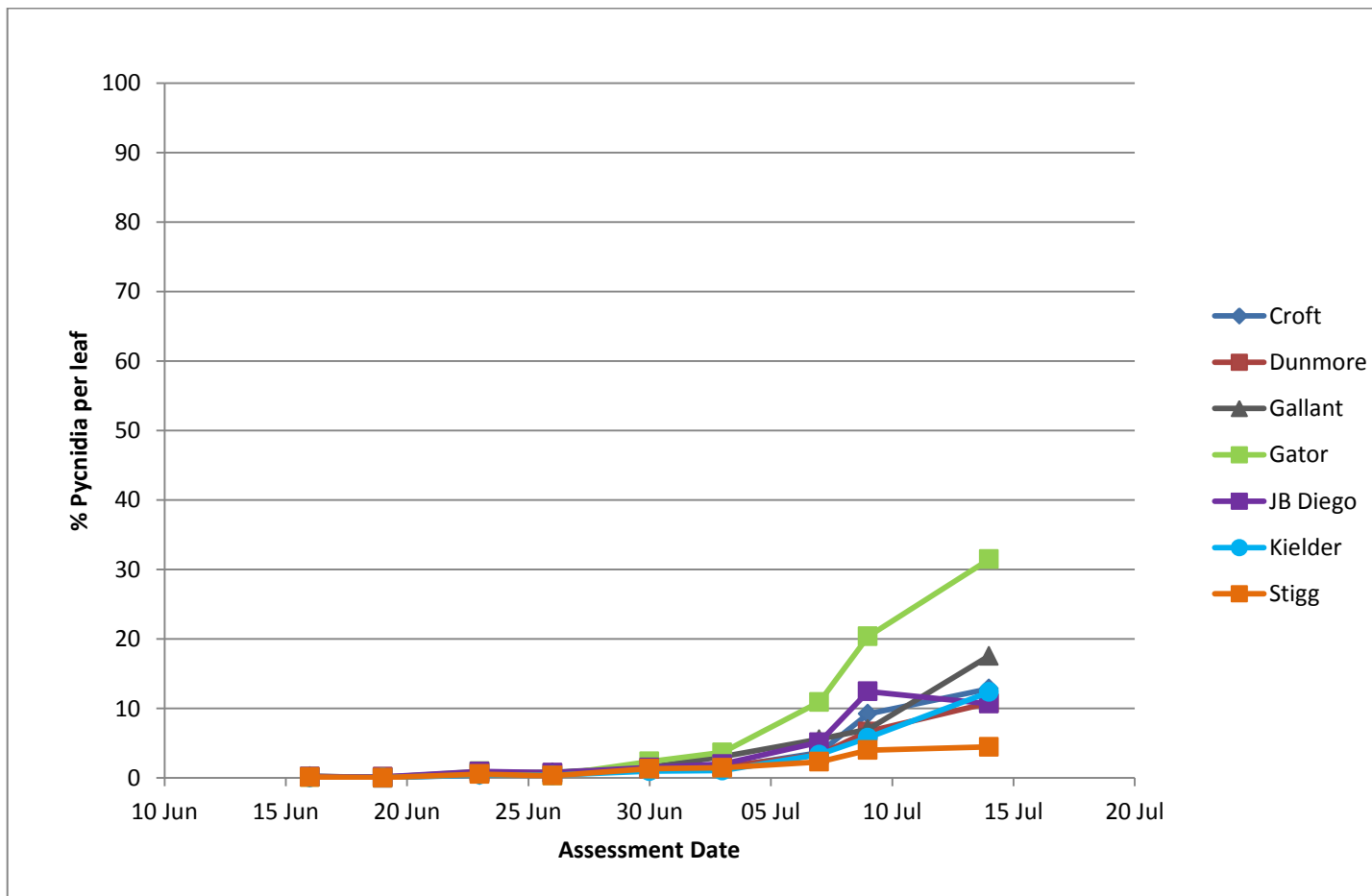


Figure 3.18: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Norwich in 2015.

During the 2015-2016 growing season the Norwich trial was limited to three disease assessment dates (12th July, 15th July and 19th July). Taking this into account there was significant differences ($P < 0.001$) between varietal performance with cv. Gallant increasing from 25% to 80% disease within 4 days. Cultivar Stigg remained consistent with disease severity only reaching 5% by the last assessment date. This level of resistance was followed closely by cv. Dunmore reaching a disease high of 20% by the final assessment date. The remaining cvs Croft, JB Diego, Kielder and Gator again showed moderate levels of disease resistance reaching between 35 and 40% by the 19th July. (Figure 3.19)

In comparison to the disease severity at approx. the same time in 2015 there was no significant difference within the 7 winter wheat varieties. Cultivar Stigg was showing 0% disease on 12th and 15th July 2016, progressing to 5% by the final assessment date (19th July). This cv. followed a similar trend in 2015 reaching 5% disease severity on 9th July and remained at this rate on the final assessment date (14th July). In 2014 it showed a slight difference reaching 20% disease by the final assessment date (11th July). Cultivar Gallant had 25% disease on both 12th and 15th July 2016 and progressed at a greater rate to 80% by the final assessment date 4 days later. In 2015 cv. Gallant followed a similar trend reaching only 20% by 14th July.

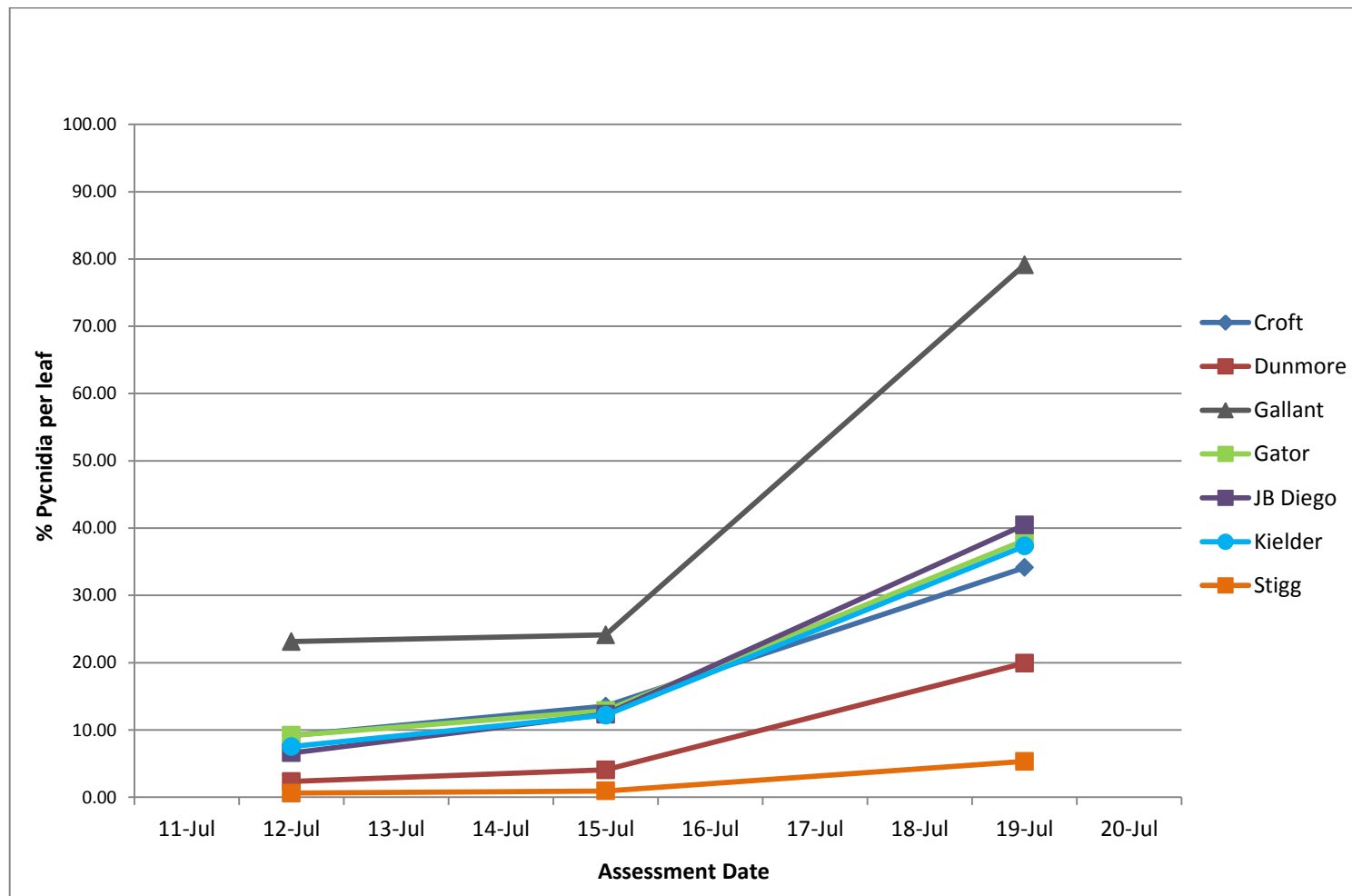


Figure 3.19: Covered by pycnidia as a percentage area of leaf area on the flag leaf in Norwich in 2016

3.5.4 Latent period of *Zymoseptoria tritici* on winter wheat

Data was recorded in Oak Park when the 2nd leaf and flag leaf started to emerge in cumulative days from the first assessment date (30th April 2015) and the time, also in cumulative days, when more than 50% of the tagged plants were bearing pycnidia (Figure 3.20). Cultivars Stigg and Kielder had the longest approximated LP on the 2nd leaf of 25.0 days and cv. Gator had the shortest approximated LP of 16.2 days. Cultivars Dunmore, JB Diego, Croft and Gallant had LP's ranging from 19.4 to 22.0 days. On the flag leaves, the LP lengthened across all cultivars with cv. Stigg and cv. JB Diego then having the longest LP's of approximately 38.0 days. Cultivar Gallant recorded the shortest approximated LP of 27.4 days with cvs Dunmore, Croft, Gator and Kielder grouping together within a range of LP's from 31.0 to 35.4 days (Figure 3.20).

Corresponding data was recorded in Waterford with the first assessment date being the 29th April 2015 (Figure 3.21). Cultivar Stigg remains the cv. with the longest approximated LP on both the 2nd leaf (27.6 days) and flag leaf (34.8 days). On the 2nd leaf cvs Kielder (16.6 days), JB Diego (17.0 days) and Gator (18.4 days) had the shortest LP's. On the flag leaf cv. Kielder remained the cultivar with shortest LP of 15.0 days along with cv. Gallant which showed an LP of 15.2 days. The remaining cultivars of Dunmore, Croft, JB Diego and Gator had a LP range from 21.0 to 23.6 days (Figure 3.21).

3.5.5 Variance of latent periods

The *Z. tritici* LP in each winter wheat variety varied between the glasshouse and field environment (Table 3.7) with the most obvious variation being in cv. Gallant which had an LP of just 12 days under glasshouse conditions and this was increased to approx. 21 days in the field environment. Cultivar Stigg displayed a longer LP in Waterford (27.6 days) than in Oak Park (25.6), this followed true for cv. JB Diego with 24.8 days in Waterford and 21.0 days in Oak Park. This was also seen with cvs Gator and Gallant.

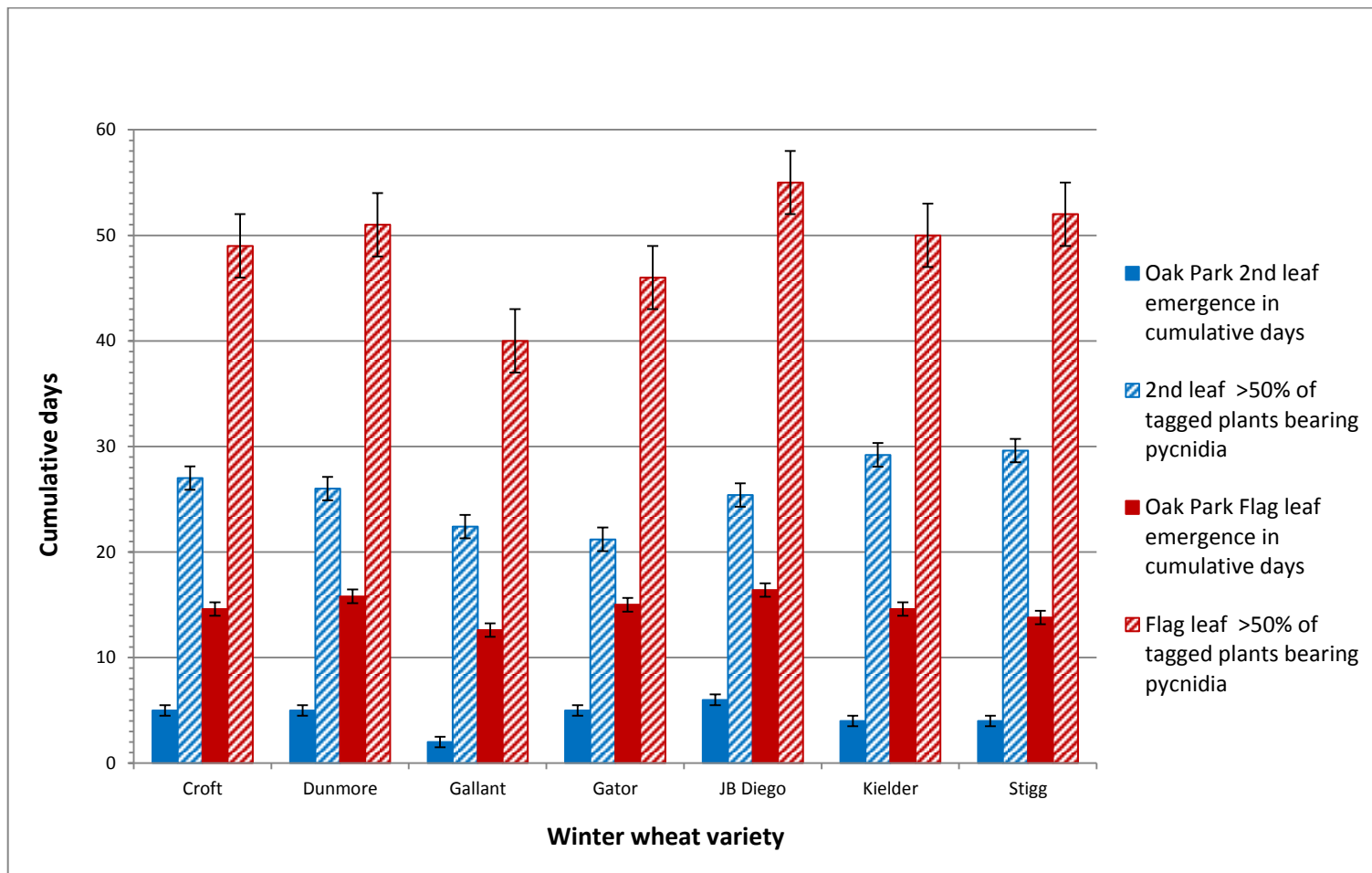


Figure 3.20: Latent Period in Oak Park on flag leaf and 2nd leaf in cumulative days from first assessment date (30th April 2015), error bars on flag leaf emergence are +/- 0.64 days, more than 50% bearing pycnidia +/- 3.0 days and error bars on 2nd leaf emergence are +/- 0.5 days, more than 50% bearing pycnidia +/- 1.1 days

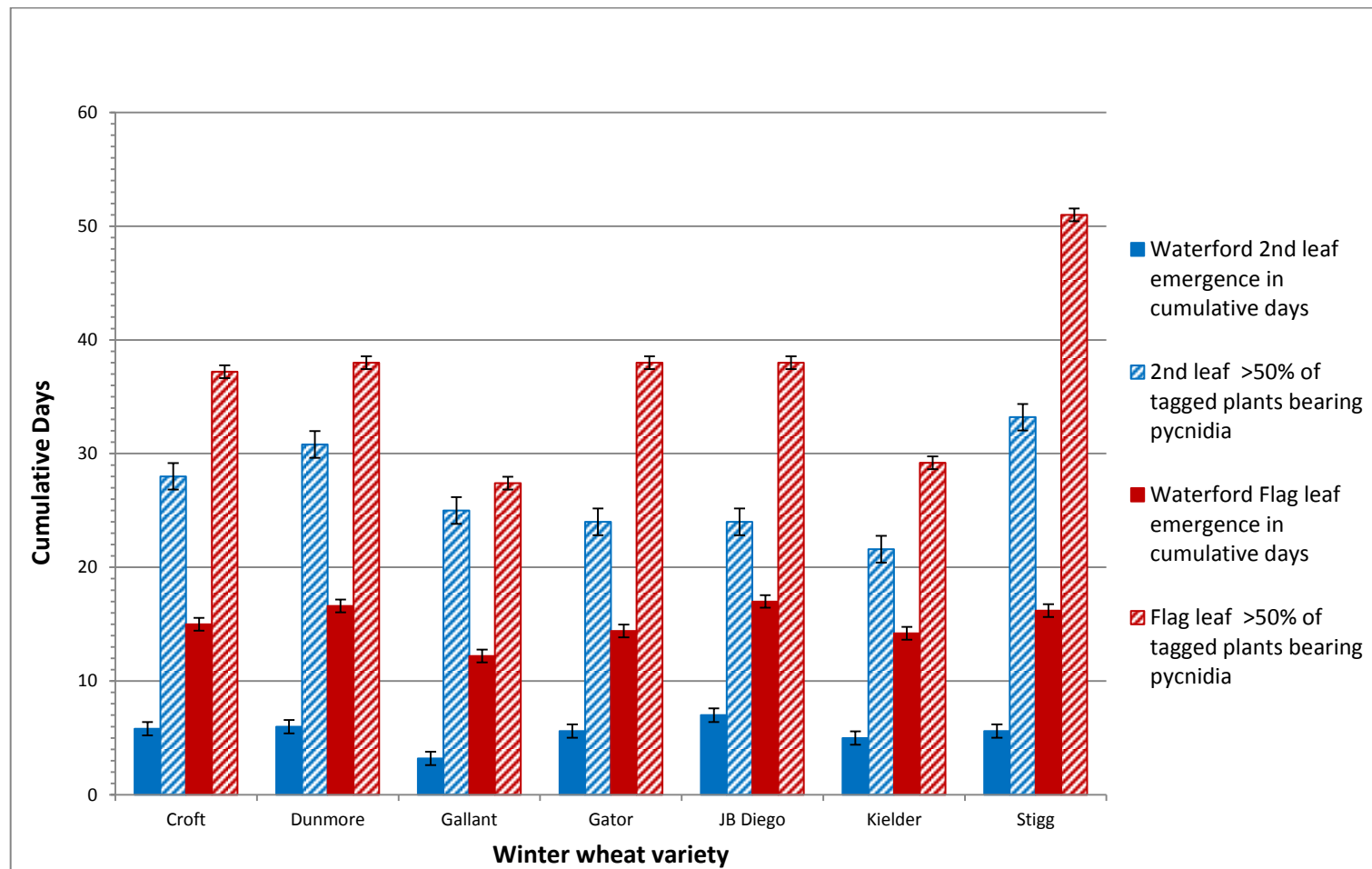


Figure 3.21: Latent Period in Waterford on flag leaf and 2nd leaf in cumulative days from first assessment date (29th April 2015), error bars on flag leaf emergence are +/- 0.56 days, more than 50% bearing pycnidia +/- 0.56 days and error bars on 2nd leaf emergence are +/- 0.59 days, more than 50% bearing pycnidia +/- 1.17 days

Table 3.7: Variance of latent periods between glasshouse and field environments

Winter Wheat Variety	LP (days) on 2 nd leaf in glasshouse	LP (days) on 2 nd leaf in Waterford	LP (days) on 2 nd leaf in Oak Park
Stigg	24.17	27.6	25.6
Dunmore	21.20	17.0	19.4
JB Diego	21.14	24.8	21.0
Croft	17.20	16.6	25.2
Gator	16.79	18.4	16.2
Kielder	16.72	22.2	22.0
Gallant	12.00	21.8	20.4

3.5.6 Yield results

Cultivar Gallant was excluded from the initial yield data analysis in Waterford, Oak Park and Norwich as it was not included in the 2014 field trials. The analysed data from the remaining six varieties (Stigg, Dunmore, Croft, JB Diego, Kielder and Gator) showed significant differences ($P=0.01$) in yields (t/ha) across all three years (2014, 2015 and 2016) in Waterford, cv. Stigg produced the highest yields; cv. Gator produced the lowest yield in 2014 and cv. Kielder in 2015 and 2016. There was a highly significant effect of year on yield ($P<0.001$) between varieties but no significant interaction ($P=0.6$) between varietal yield and year (Figure 3.22).

The data was re-analysed for 2015 and 2016 only to include cv. Gallant and the results showed there was no significant difference ($P=0.52$) between the two years within varieties but there was high significant difference ($P<0.001$) between varieties in both years. Cultivar Stigg showed significant difference (l.s.d = 2.140) in both years to cvs Gallant, Gator and Kielder (>2.140), significant difference to cvs Croft and Dunmore in 2016 (≥ 2.140) but no significant difference in 2015 (<2.140). However, cv. Stigg showed no significant difference to cv. JB Diego in both years (<2.140). Cultivar Gallant showed significant difference to cvs JB Diego, Dunmore and Kielder in 2015

(>2.140) but no significant difference in 2016 (<2.140). Gallant also showed no significant difference to cvs Kielder and Gator in both years (Figure 3.22)

The analysed data for Oak Park excluding cv. Gallant showed significant differences ($P=0.03$) between yields (t/ha) across all three years. There was high significant difference ($P<0.001$) between varieties across the three years and also a significant interaction ($P=0.001$) between varieties and years, as the varieties' yields ranked differently in the three years. The re-analysed data for 2015 and 2016 in Oak Park including cv. Gallant showed slight significant difference ($P=0.03$) between 2015 and 2016 in cvs Stigg and JB Diego but no significant difference ($P=0.08$) between the other five varieties (Dunmore, Croft, Kielder, Gator and Gallant). Oak Park also observed high significant difference ($P<0.001$) between varieties in both years of the trials (Figure 3.23).

In addition to cv. Gallant, cvs Kielder and Gator were also excluded from the initial yield data analysis in Norwich as they displayed very poor establishment rates in 2014 and could not be included in the experiment. The analysed data from the remaining four varieties (Stigg, Dunmore, Croft and JB Diego) showed high significant differences ($P<0.001$) between yields (t/ha) across all three years (2014, 2015 and 2016). There was significant effect of year ($P=0.008$) between varieties between all three years and high significant difference ($P<0.001$) within variety between years (Figure 3.24).

The data was re-analysed for 2015 and 2016 only to include cvs Gallant, Kielder and Gator and the results showed there was no significant difference ($P=0.464$) between the two years and also no significant difference ($P=0.084$) within varieties between years. Fisher's protected LSD was not calculated as the variance ratio for variety was not significant ($P=0.161$).

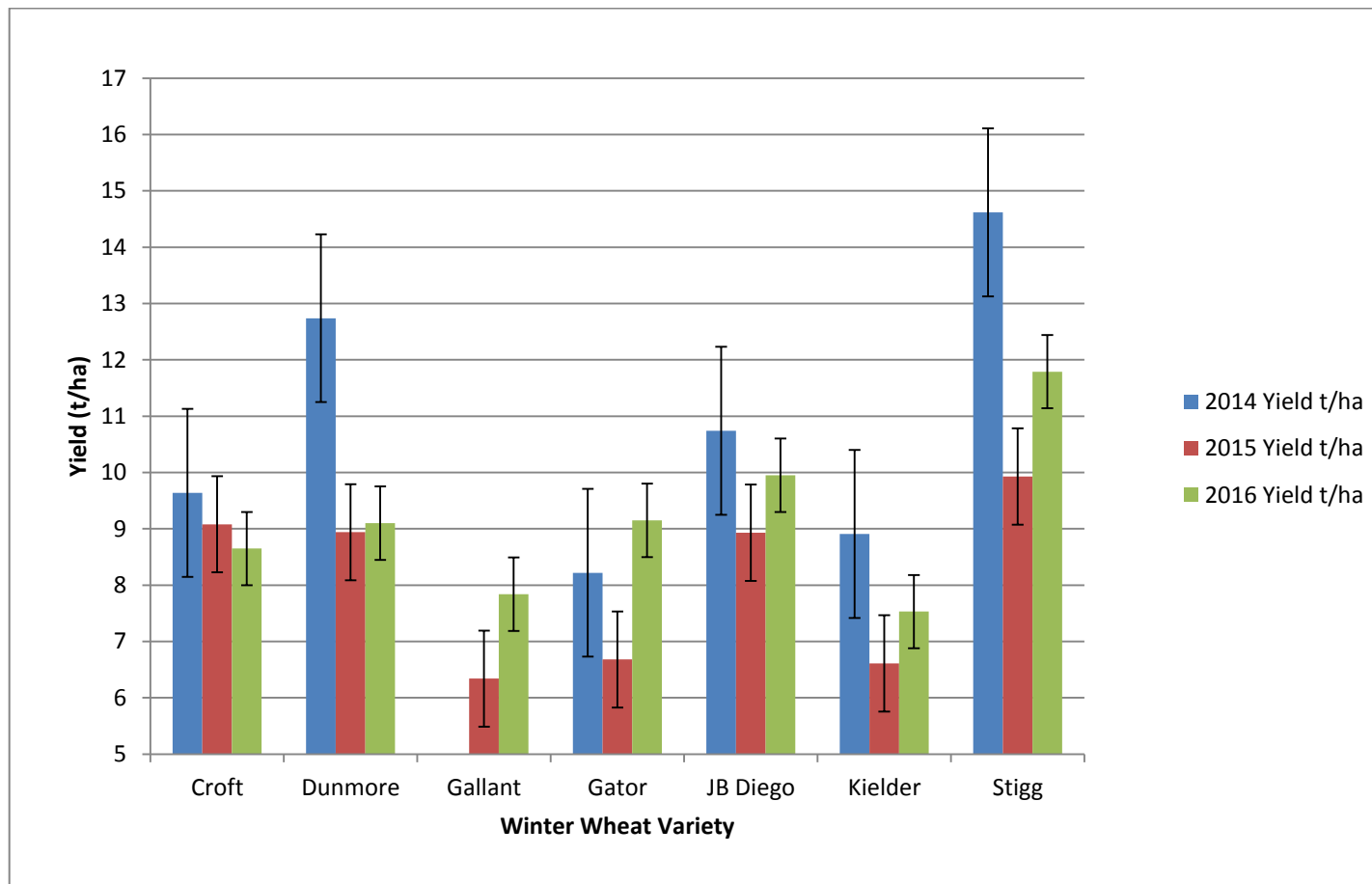


Figure 3.22: Mean varietal yields (t/ha) across all three growing seasons in Waterford. Error bars 2014 (+/- 1.5 t/ha), 2015 (+/- 0.85 t/ha), 2016 (0.65 t/ha).

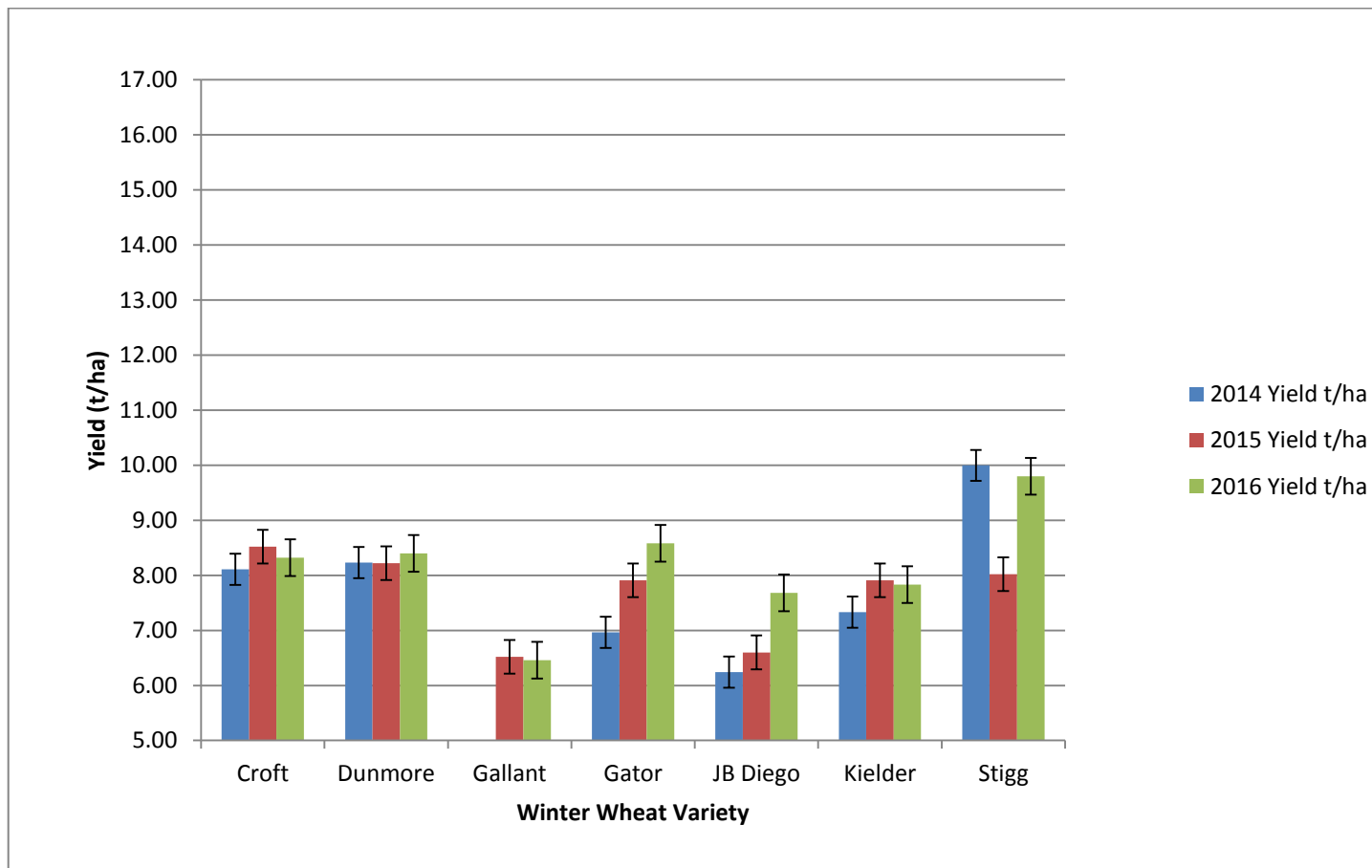


Figure 3.23: Mean varietal yields (t/ha) across all three growing seasons in Oak Park. Error bars 2014 (+/- 0.28 t/ha), 2015 (0.31 t/ha), 2016 (0.33 t/ha).

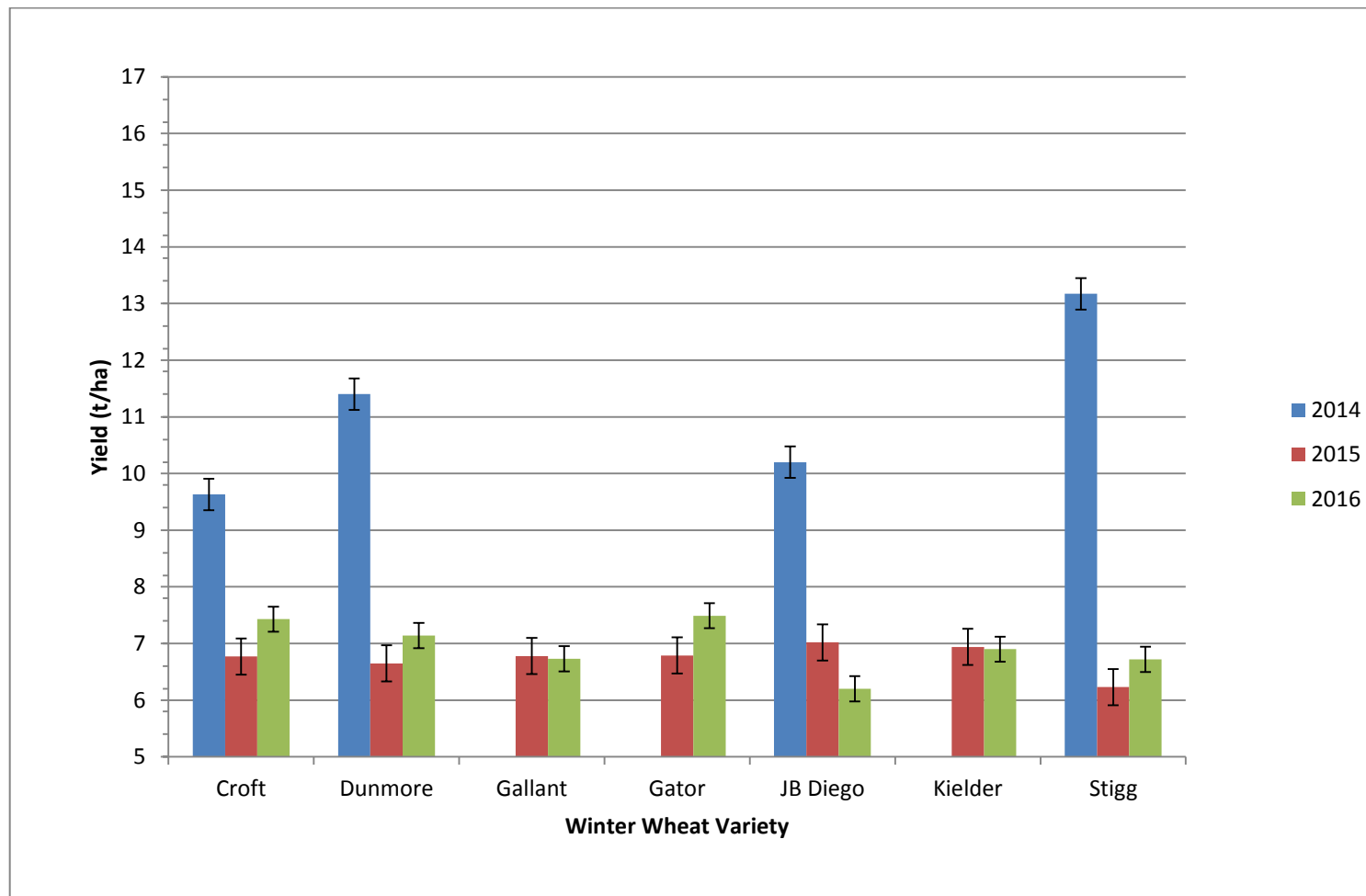


Figure 3.24: Mean varietal yields (t/ha) across all three growing seasons in Norwich. Error bars 2014 (+/- 0.28 t/ha), 2015 (0.32 t/ha), 2016 (0.22 t/ha).

3.6 Discussion

The research reported in this study identified substantial variation in the *Z. tritici* latent period and the rate of the fungal pathogen's disease progression between seven winter wheat cultivars in three field environments (high, medium and low inoculum pressure). The respective climates of the three trial sites contributed to the variation in the dates when disease first appeared on the leaves, disease progression rates throughout the growing seasons and also the approximate length of the latent period between cultivars. The LP is similar across all 7 winter wheat varieties tested but the rate of disease progression is subsequently slower in cv. Stigg (Figure 3.20 and Figure 3.21).

The results of these trials represented the climate for the particular year of assessments; it is for this reason that there is a necessity to conduct field trials which study disease progression in a range of environments and also over a minimum of three years to collect relative and accurate data. Based on disease scores, temperature, rainfall, humidity and inoculum pressure, varieties may be identified as partially resistant or susceptible in a particular growing season. This was seen in cv. Kielder at the high inoculum pressure trial site in Waterford when it showed an increased susceptibility to Septoria disease in 2015 in comparison to 2014 and 2016. This was potentially due to the climate conditions during the 2015 growing season when the months of May and July saw over double the millimetres of rainfall than in the other years of the trials, allowing for the rain splash dispersal of spores from the lower leaves in the crop canopy to the newly emerging leaves (Thomas *et al*, 1989.; Shaw, 1990.; Shaw & Royle, 1993.; Suffert *et al*, 2010). The rapid progression of disease from the 7th to the 17th July was quite likely due to ideal growth conditions for Septoria (Eyal, *et al*, 1987.; Shaw, 1990) during the month of July 2015 when the mean temperature was 14°C and there was a total rainfall of 116mm (data taken from the nearest weather station (43km) to trial site in Waterford which is Johnstown Castle AWS, County Wexford, Latitude: 52.292, Longitude: -6.491).

During the 2013-2014 growing season at the medium inoculum pressure trial site in Oak Park, there was a slight decrease in disease percentage on the 2nd leaf of cv. Stigg between two of the assessment dates (2nd and 4th July) (Figure 3.10). Slight increase, if any, in disease severity can also be seen on the flag leaves across all varieties between the same dates (Figure 3.11). Cultivar Stigg recorded an increase of just 0.6% and cv. Gator had 0% increase. This indicates that at this stage in the growing season disease progression rates are more obvious on the 2nd leaf of the plants than on the flag leaves with the exception of cv. Stigg possibly because of its higher partial resistance to *Z. tritici*.

The reason for low % disease during the 2014-2015 growing season at the low inoculum pressure trial site in Norwich may have been due to a decrease in rainfall during the month of May from 94mm in 2014 to 47mm in 2015 (weather data taken from the Lowestoft, Monckton Ave weather station by the British Meteorological Office). The Norwich trial also recorded a drop in disease severity on cvs Croft (2nd leaf) and JB Diego (2nd and flag leaf), on different assessment dates. The reason for this fluctuation in the dataset is also due to various people carrying out the assessments and the individual's perception of *Z. tritici* disease as a percentage. Variation between scorers was minor (+/- 5% pycnidia on the leaf) and did not have a substantial effect on the conclusions in relation to the varying disease progression rates within the cultivars.

To eliminate these errors in the datasets it is important that when possible the same individual should carry out all assessments throughout the growing season. Performance of the 7 winter wheat varieties tested was as expected across all three environments with cv. Stigg displaying the highest level of partial resistance to *Z. tritici*, followed by cv. Dunmore and cv. Gallant displaying the most susceptibility to infection of the fungal pathogen. The performance of the 3 different field environments was also as expected with Waterford displaying the highest levels of *Z. tritici* disease, followed by Oak Park 2 weeks later and finally Norwich consistently displaying the lowest levels of infection throughout the growing seasons.

Variations in the *Z. tritici* LP (Table 3.11) between glasshouse and field environments are more than likely due to the glasshouse experiments being artificially inoculated with the Dutch *Z. tritici* isolate IPO323 and incubated under

controlled environment conditions whereas the field experiments were infected naturally by the wild-type isolates present in the respective environments and there was no control over the temperature, humidity, light or inoculum density.

The LP may also be dependent on the amount of inoculum present on the leaf surface and also in the field trials, did the inoculum present in the environment, infect as soon as leaf emergence occurred? For this reason, it was important to test the different winter wheat varieties and their interactions with *Z. tritici* under controlled and uncontrolled environment conditions to fully understand the impact that the environment has on the LP of *Z. tritici* and also the rate at which the fungal pathogens disease severity progress. Cultivar Stigg had an LP of 24.2 days under controlled glasshouse conditions and an initial inoculum density of 1×10^7 , in the uncontrolled field environment it had an approximate LP of 27.6 days (high inoculum pressure) and an approximate LP of 25.6 days (medium inoculum pressure). The highly susceptible cv. Gallant had an LP of 12 days in the glasshouse and an approximate LP of 21.8 days (high inoculum pressure) and 20.4 days (medium inoculum pressure) in the field environment. Cultivars JB Diego and Gator showed LP's of a similar manner, where the high inoculum pressure environment LP was longer than the medium inoculum pressure LP. The other three cultivars (Dunmore, Croft and Kielder) showed shorter LP's in the high inoculum pressure environment as expected. This data has previously been tabulated earlier in this chapter in Table 3.7. This data reinforces previous research into the effects of temperature, humidity and inoculum density within the crop canopy (Shaw, 1990.; Shaw & Royle, 1993) and re-emphasises that although airborne inoculum is a major contributor to Septoria epidemics, other environmental factors play a major role in the infection process of *Z. tritici* leading to Septoria events.

Yield data recorded from harvesting the three years of field experiments (Figures 3.22, 3.23 and 3.24) indicates the varying yield potential of winter wheat varieties in the different environments between years and also the difference in yields (t/ha) between varieties. The harvest of 2015 trials in Waterford and Oak Park showed lower yields than in 2014 and 2016. In 2014 the Norwich site recorded the highest mean yields (11.10 t/ha), Waterford recorded higher mean yields (10.81 t/ha) than Oak Park (7.57 t/ha). This is further confirmation of the importance of testing the 7 winter wheat varieties in

different environments and how environmental factors can contribute to disease severity and effect yield potential.

As with the nature of field studies, environmental and logistical challenges were encountered. The intensive data collection in Waterford and Carlow consisted of one scorer recording 5600 (20 tagged plants x 35 plots x 2 leaves x twice weekly x two trial sites) visual assessments per week. The sheer amount of labour involved in this was not always possible for one person and so variation between scorers very slightly influenced the collected data. To reduce the variation between scorers, the previous days collected data was given as guide. The same quantity of collected data was not possible at the Norwich trial site, which had 10 tagged plants x 35 plots x two leaves x once per week.

Although fungicide applications may be more effective when applied at the optimal time in relation to the latent period, experience from this research, brings to light the difficulties involved in gaining an accurate measurement of LP. It may be questioned, if the benefit of more accurate timing could be outweighed by the cost of collecting the required data.

Future research may benefit from advances in technology. For example, could drones be used in future to score trials more accurately and efficiently than a person? Could advances in image analysis software allow for distinguishing between the yellowish–brown lesions of STB disease and the brown soil or pale green leaves?

There are still many areas which require further research to aid in the worldwide struggle against this devastating disease in Winter Wheat.

Chapter 4: Quantifying the rate of fungal growth in winter wheat cultivars during the *Zymoseptoria tritici* latent period

4.1 Introduction

The general method for quantification of disease presence is visual assessment as a percentage of the leaf area covered in disease; however this method is subjective and relies on the consistency of the observer and simplicity to reduce the time involved in assessments (Cooke, 2006). An alternative to visual assessment is to quantify pathogen levels through the quantification of total DNA by quantitative PCR (qPCR) to compare with visual assessments. The use of qPCR to detect a pathogen's presence in wheat has been demonstrated for a range of starting materials such as soil, grain and leaves (Li *et al.*, 2013.; Nicolaisen *et al.*, 2009.; Bearchell *et al.*, 2005.; Guo *et al.*, 2006). General guidelines have been published for reporting qPCR by Bustin *et al.*, 2009; however various methods have been used to express the detected pathogen DNA (standardised pathogen DNA by total extraction (Fraaije *et al.*, 1999), wet or dry sample weights Reischer *et al.*, 2004)) while other studies do not report the method used for standardising (Guo *et al.*, 2006).

Previous studies have developed qPCR assays for the detection of *Z. tritici* using the β -tubulin gene as the target (Guo *et al.*, 2006.; Fraaije *et al.*, 1999.; Bearchell *et al.*, 2005) but still very little is known in terms of fungal biomass present during the latent period, particularly across a range of winter wheat cultivars. Fraaije *et al.*, used a PCR/PicoGreen assay to detect the presence of *Zymoseptoria tritici* in artificially infected wheat leaves and did not detect an exponential increase in fungal biomass until 14 days post-inoculation. Bearchell *et al.*, (2005) used qPCR assays to determine the amounts of *Z. tritici*, *Parastagonospora nodorum* and wheat DNA present in archived samples spanning a 160 year period of wheat production using a 63-bp fragment of the β -tubulin gene (GenBank accession no. AY547264) as the target. Guo *et al.*, (2006) also used a qPCR assay to monitor the development of *Z. tritici* in

inoculated and naturally infected wheat plants, reporting the rapid growth period to be between 6 and 16 days after inoculation. This growth period is faster than previously reported by Fraaije in 1999 which may have been due to slightly different controlled environment cabinet conditions and inoculum concentrations. Fraaije *et al.* (1999) inoculated seedling plants at GS11 with two droplets of 6µl (10^5 spores ml⁻¹) and placed the seedlings in two controlled environment cabinets, set at 12 and 18°C with 12h/12h light/dark alternation. Guo *et al.* (2006) inoculated 40 day old seedlings by spraying them with an increased concentration of 10^7 pycnidiospores ml⁻¹ conidial suspension and placing them in a growth cabinet set at 22/17°C day/night and a photoperiod of 12h.

The aim of this research was to improve knowledge of the latent period of *Septoria tritici* blotch in wheat by (i) establishing a qPCR diagnostic assay following Guo *et al.* (2006) and (ii) applying this assay to quantify the rate of *Z. tritici* fungal biomass accumulation in wheat cvs with varying LP's and field susceptibility following controlled inoculation under glasshouse conditions and natural infection of field grown wheat.

4.2 Materials and methods

4.2.1 Standard curves, limit of detection, quantification efficiencies and optimised qPCR assay.

Each qPCR assay included three replicates of each sample (technical replicates) of non-template controls (NTC, i.e. water) and standardised DNA concentrations (50000pg/µl – 0.05pg/µl for *Z. tritici* isolate 396.12, 30000pg/µl – 0.03pg/µl for *Z. tritici* isolate IPO323 and 40000pg/µl – 0.04pg/µl for *T. aestivum*) diluted 10-fold. Each qPCR assay was carried out on a Roche LightCycler® 96 System (Roche Diagnostics GmbH, Mannheim, Germany) in 20µl reaction volumes consisting of 1µl total DNA sample, 10µl Fast start Essential DNA Probes Master (Roche), 0.75µl FC 375nM Universal probes labelled with FAM (Roche), 0.5µl left primer FC 250nM, 0.5µl right primer FC 250nM (Sigma Aldrich) and 7.25µl of molecular grade water (Sigma Aldrich).

Reactions were run for 10 minutes at 95°C pre-incubation followed by 40 cycles of 10s at 95°C, 30s at 60°C and 10s at 72°C. The increase in fluorescence from probes was registered at every temperature step and cycle during the reaction and data was analysed by the instrument's software. For each sample the quantitative cycle (Cq) value was determined. Standard curves for each of the target genes were generated by plotting the log concentrations of a 10 fold standard dilution series against the Cq values.

To quantify the *Z. tritici* DNA present in the leaf samples, quantitative PCR was carried out targeting the *Z. tritici* β -tubulin gene. The target sequence for the field study samples was obtained from the *Z. tritici* tub1 (AJ310917.1) gene for beta-tubulin (Fraaije et al., 2001), and for the glasshouse study from the *Z. tritici* strain IPO323 beta-tubulin (tub) (JF700993.1) gene (Quaedvlieg et al., 2011). Two β -tubulin genes were used as following further research, it was discovered the *Z. tritici* β -tubulin probe and primers did not detect the presence of *Z. tritici* strain IPO323 in the artificially inoculated glasshouse samples. When both sequences were aligned, it was discovered that the β -tubulin gene in *Z. tritici* strain IPO323 had a slight difference in its DNA sequence to the previously used target sequence in *Z. tritici* (Figure 4.1). The primers and probe to detect the presence of *Z. tritici* strain IPO323 were redesigned using Genbank accession number: JF700993.1 (Quaedvlieg et al., 2011). Data returned was then normalised against two *T. aestivum* reference genes, α -tubulin (U76558.1) (Segal & Feldman, 1996) and GAPDH (Piattoni et al, 2007, unpublished. GenBank accession no. EF592180.1) (Table 4.1).

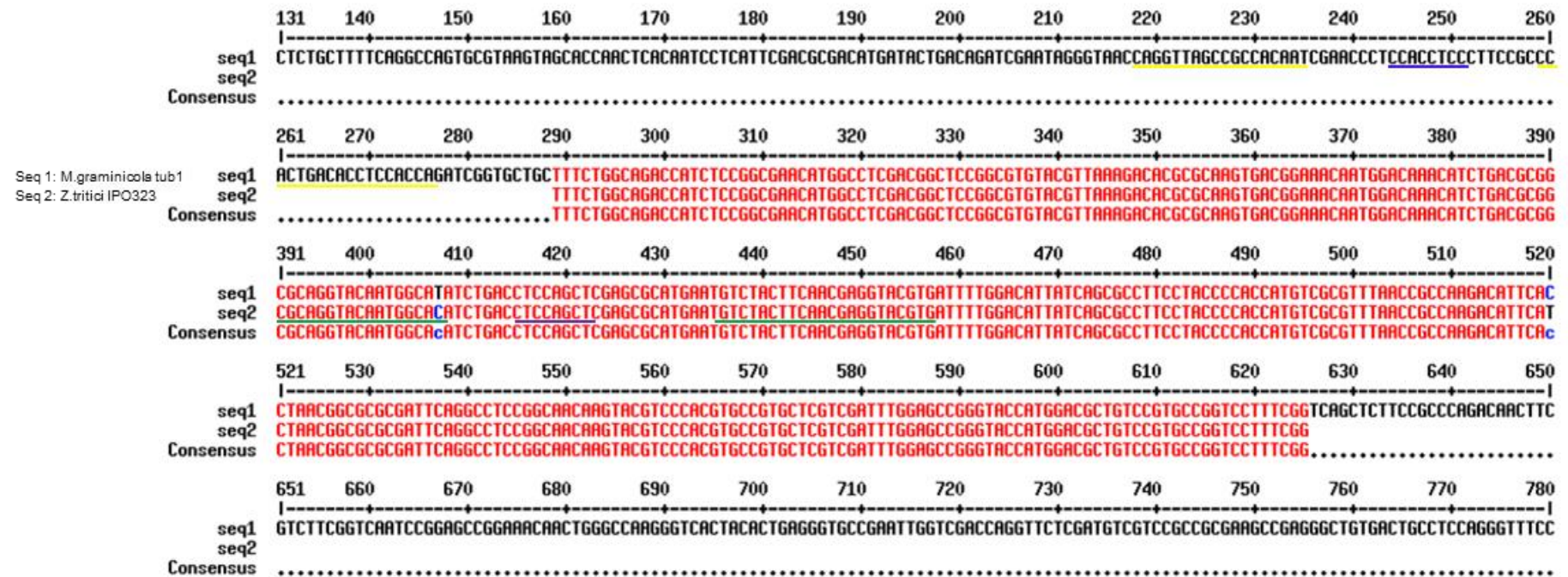


Figure 4.1: *Zymoseptoria tritici* β -tubulin tub1 gene GenBank: AJ310917.1 (primers forward and reverse underlined in yellow, probe underlined in blue) and *Zymoseptoria tritici* strain IPO323 β -tubulin GenBank: JF700993.1 (primers forward and reverse underlined in green and probe underlined in purple) sequence alignment. Difference in DNA sequences highlighted in blue.

Table 4.1: Quantitative Polymerase Chain Reaction (qPCR) probe and primers used to amplify both the β -tubulin genes in *Z. tritici* and *T.aestivum* genes α -tubulin and GAPDH.

Probe / Primer	Sequence	Amplicon size (bp)
<i>Z. tritici</i> (formally <i>M. graminicola</i>)		
tub1 gene GenBank: AJ310917.1		
<i>Z.tritici</i> β -tubulin Probe	CCACCTCC	8
<i>Z.tritici</i> β -tubulin Left Primer	CAGGTTAGCCGCCACAAT	18
<i>Z.tritici</i> β -tubulin Right Primer	CCACTGACACCTCCACCAG	19
<i>Z.tritici</i> strain IPO323		
GenBank: JF700993.1		
<i>Z.tritici</i> β -tubulin Probe	CTCCAGCT	8
<i>Z.tritici</i> β -tubulin Left Primer	CGCAGGTACAATGGCACA	18
<i>Z.tritici</i> β -tubulin Right Primer	CACGTACCTCGTTGAAGTAGACA	23
GenBank: U76558.1		
<i>T. aestivum</i> α -tubulin Probe	CTGCCTCC	8
<i>T. aestivum</i> α -tubulin Left Primer	GGTGATGAGGGCGATGAG	18
<i>T. aestivum</i> α -tubulin Right Primer	CAGTAGCAAGCCTTGGGAAA	20
GenBank: EF592180.1		
<i>T. aestivum</i> GAPDH Probe	GCCAAGAA	8
<i>T. aestivum</i> GAPDH Left Primer	CAAGGCTGCAGCTCACATTA	20
<i>T. aestivum</i> GAPDH Right Primer	CCTTGCTGGGAGCAGAAAT	19

4.2.2 Glasshouse study: Investigating the rate of *Zymoseptoria tritici* biomass accumulation in cvs Stigg and Gallant, representing strong partial resistance and highly susceptible wheat phenotypes.

Two consecutive replicates of a time course trial on cvs Stigg and Gallant were conducted in February/March 2016 and March/April 2016. The trials were laid out in a randomised design (Figure 4.2) generated using Microsoft Excel's randomisation function. The first and second leaf of 168 individual 2-3 week old seedling plants were inoculated with a spore suspension of *Z. tritici* Dutch isolate IPO323 (Figure 4.3) at a concentration of 1×10^7 spores/ml with 2-3 drops of Tween20 in 2000ml. Another 168 individual negative controls were also inoculated in the same manner with a suspension of water and Tween20. All seedlings were sprayed until run-off. Plants were covered with plastic bags for 48h to allow for leaf penetration (Fraaije *et al.*, 1999; Kema *et al.*, 1996a). The second leaf from each individual plant was harvested into liquid nitrogen at 24 hour intervals from 0 to 17 days after inoculation (dai) and also at 20, 24 and 28 dai. Harvested leaf material was then stored in a -80°C freezer until a time when DNA could be extracted. All plant leaves other than the inoculated 1st and 2nd leaves were kept clipped in order to facilitate light penetration and disease assessment of target leaves as described by Kema *et al.*, 1996a.

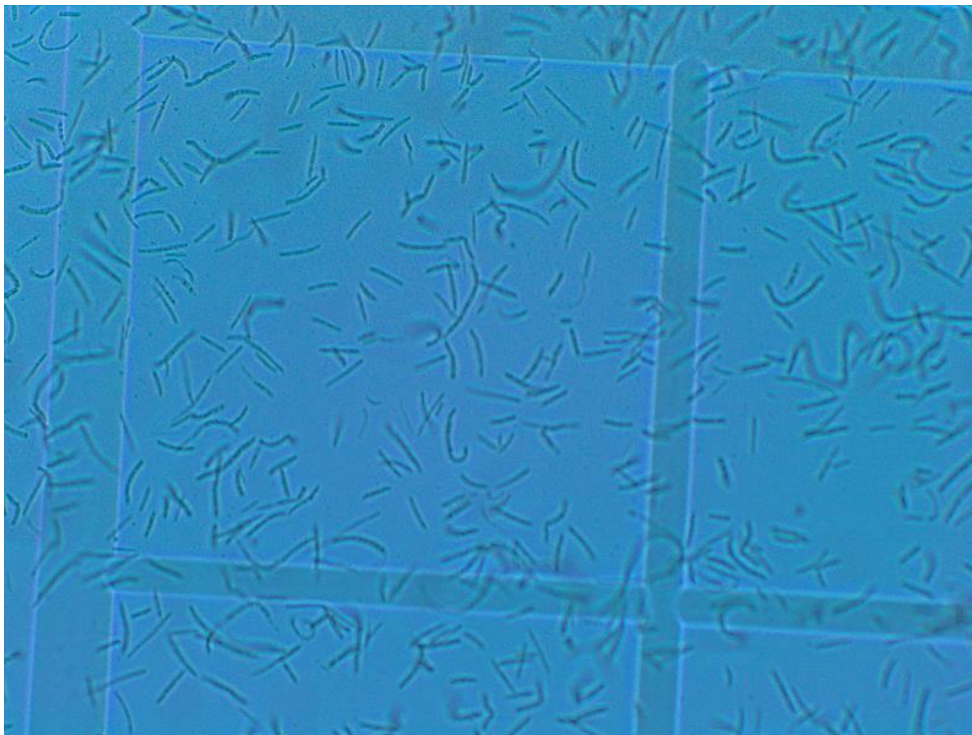
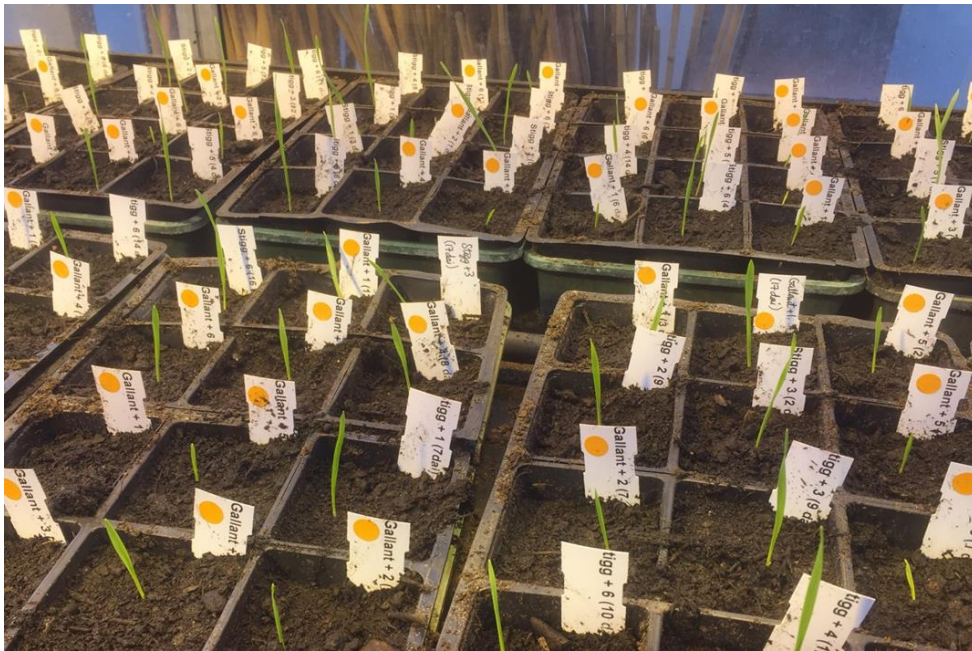


Figure 4.3: *Zymoseptoria tritici* Dutch isolate IPO323 spores viewed by haemocytometer 1000X

4.2.3 Field study: Investigating the rate of *Zymoseptoria tritici* biomass accumulation in 7 winter wheat cultivars, representing strong / moderate partial resistance and highly susceptible wheat phenotypes.

To correspond with the visual assessments of % disease progression leaves which were visually displaying high or low symptoms of Septoria Leaf Blotch (STB) were sampled from the Oak Park field trial previously described in detail in Chapter 3 (Figure 4.4) at three time points throughout the 2014/2015 growing season (8th May, 26th June and 8th July) and the fungal biomass quantified by qPCR. Six fully emerged leaves from main tillers were sampled from each of the 35 plots (7 wheat varieties x 5 reps). The 7 winter wheat varieties sampled were cv. Stigg, cv. Dunmore, cv. JB Diego, cv. Croft, cv. Kielder, cv. Gator and cv. Gallant. STB was allowed to develop naturally by applying fungicide products to control yellow rust and mildew which had minimal effect on STB.

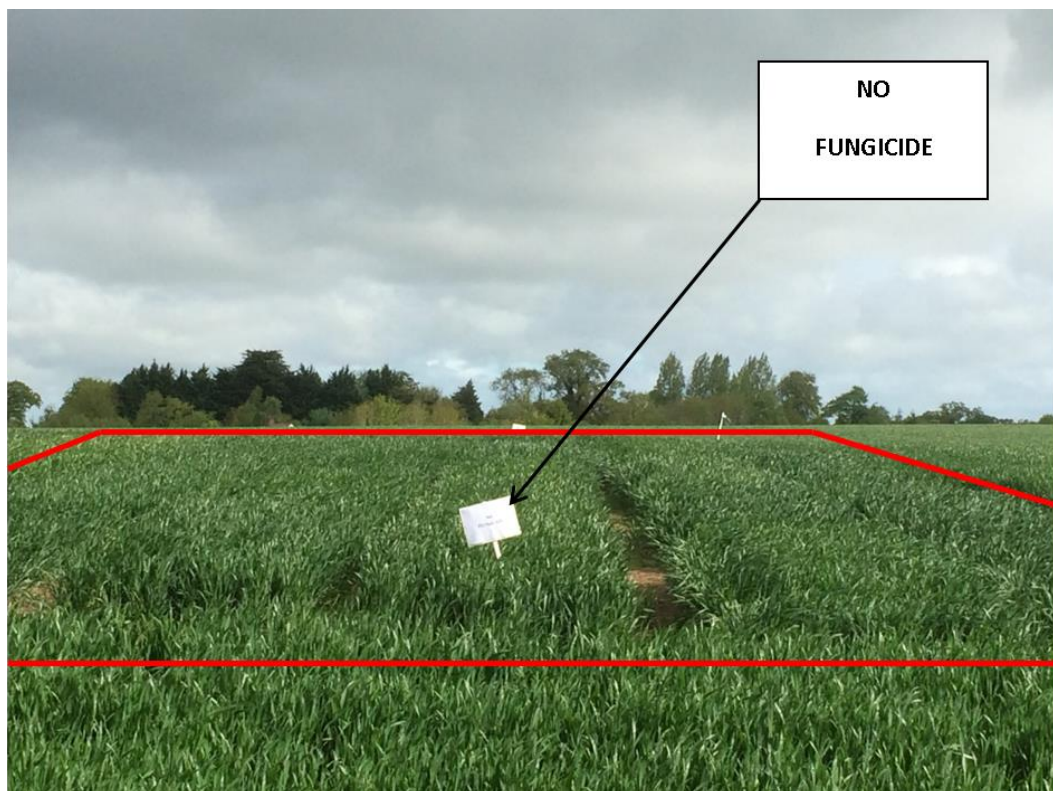


Figure 4.4: Oak Park trial site 2014-2015

4.2.4 Sample storage and DNA extraction

Quality and quantity of the extracted DNA affect the efficiency of molecular techniques such as qPCR (Varma *et al.*, 2007). The 6 leaves sampled from each of the 35 plots in the field study were combined in a large mortar and ground with liquid nitrogen using a pestle to disrupt the plant cell wall. A sub-sample (~100mg) was taken from each of the 35 samples and the total DNA was extracted using the Sigma Aldrich GenElute Plant genomic DNA extraction kit following the manufacturers recommended protocol (SigmaAldrich, 2010). DNA extraction protocol: 350µl lysis solution, part A containing guanidine thiocyanate (chaotropic salt which destabilises hydrogen bonds) + 50µl lysis solution, part B containing a detergent to help solubilise and lyse cell proteins, was added to the ground leaf tissue and incubated at 65°C for 10 minutes. 130µl precipitation solution containing alcohol was added and the sample was placed on ice for 5 minutes to enhance and influence the binding of nucleic acids to silica, before centrifuging at maximum speed (16,000 x g) for 5 minutes to pellet cellular debris, proteins and polysaccharides.

The supernatant now containing the solubilised DNA was pipetted onto the filtration column and centrifuged at maximum speed for 1 minute to remove any remaining contaminating proteins, RNA or macromolecules. The filtration column was discarded and 700µl binding solution containing the chaotropic salt guanidine thiocyanate was added to the flow through liquid and mixed by inversion. This step is critical for binding the DNA to the silica binding column. 500µl of column preparation solution was added to the binding column to prepare the silica for DNA binding and centrifuged at 12,000 x g for 30 seconds to 1 minute; the flow through liquid (column prep solution) was discarded. 700µl of the filtrated solubilised DNA and binding solution mixture was then added to the binding column and centrifuged at maximum speed for 1 minute, the flow through containing any remaining impurities, proteins and polysaccharides was discarded and the binding column replaced in the collection tube, the remaining filtrated DNA and binding solution mixture was added and centrifuged again at max speed for 1 minute, the flow through once again was discarded.

The binding column now containing the bound DNA was placed in a fresh 2ml collection tube and 500µl diluted (ethanol) wash solution was added to the binding column and centrifuged at max speed for 1 minute to wash any residual proteins, salts and polysaccharides from the column, the flow through was then discarded and repeated with another 500µl of wash solution, centrifuged at max speed for 3 minutes and the flow through discarded once again. The binding column was transferred to a fresh 2ml collection tube and 100µl of pre-warmed (65°C) elution solution (10mM Tris, 1mM EDTA, pH approximately 8.0) was added to release the pure DNA from the silica, centrifuged at maximum speed for 1 minute, and the elution repeated. DNA samples were stored at -20°C for further analysis.

The extracted DNA was quantified by spectrophotometry (using NanoDrop 2000) and by agarose gel electrophoresis. This allowed the visual assessments of the percentage disease progression to also be expressed as the quantified fungal biomass (pg/µg) present in the sampled leaves. Harvested leaf samples from the glasshouse study were lyophilised in a freeze dryer for 48 hours (Labconco 6L) and then homogenised using a bench top mixer mill (Retsch Mixer Mill). The DNA was extracted as previously described.

4.3 Data analysis

Linear regression equations from the plotted standard curves of Cq value against fungal DNA concentration (pg, log) were used to calculate the concentration of DNA (*Z. tritici* pg / µg Wheat DNA) in the glasshouse and field samples by substituting the Cq values obtained from the qPCR assays into the equation of the line for each of the target genes.

4.4 Results

4.4.1. Standard curves, limit of detection, quantification efficiencies and optimised qPCR assay.

The *Z. tritici* strain 396.12 β -tubulin gene standard curve had an r^2 value of 0.99 with a slope of -3.42 (Figure 4.5) and an efficiency of 95% (Table 4.2). The *Z. tritici* strain IPO323 β -tubulin gene standard curve also had an r^2 value of 0.99 with a slope of -3.58 (Figure 4.6) and an efficiency of 98% (Table 4.2). Both *Z. tritici* standard curves consisted of 6 points with a starting concentration of 50000pg/ μ l (*Z. tritici* strain 396.12) and 30000pg/ μ l (*Z. tritici* strain IPO323) diluted in 10-fold dilutions to 0.5pg/ μ l and 0.3pg/ μ l respectively. Hence, the limit of detection (lowest concentration of *Z. tritici* detected by the instrument) in the standard curves was 0.5pg/ μ l (*Z. tritici* strain 396.12) and 0.3pg/ μ l (*Z. tritici* strain IPO323). The Roche LightCycler® 96 did not detect the lowest 10 fold dilutions tested (0.05pg/ μ l and 0.03pg/ μ l), this was most probably due to the quality of the starting DNA concentration. The *T. aestivum* α -tubulin standard curve had an r^2 value of 0.97 with a slope of -2.84 (Figure 4.7) and an efficiency of 99% (Table 4.2) and *T. aestivum* GAPDH standard curve had an r^2 value of 0.99 with a slope of -3.68 (Figure 4.8) and an efficiency of 95% (Table 4.2). Both the *T. aestivum* standard curves consisted of 5 points with a starting concentration of 40000pg/ μ l diluted in 10-fold dilutions to 4pg/ μ l. Hence, the limit of detection (lowest concentration of *Z. tritici* detected by the instrument) in the standard curve was 4pg/ μ l. The Roche LightCycler® 96 did not detect the two lowest 10 fold dilutions tested (0.4pg/ μ l and 0.04pg/ μ l), this was also most probably due to the quality of the starting DNA concentration. To validate a primer set as efficient, the set must have an amplification efficiency of >90% and the closer the slope of the standard curve is to -3.33, the closer the amplification efficiency is to the 100% ideal (SA Biosciences, 2008). An efficiency of 2.00 would equate to 100% efficient, to achieve this in a qPCR assay, the increase in fluorescence for each of the standards in the dilution series should register a Cq value every 3.33 cycles. As 2.00 is 100% efficient and a primer set cannot be 105% efficient, a value of 1.95 and a value of 2.05 both equate to 95% efficiency.

Table 4.2: Efficiency of Primers for *Z. tritici* and *T. aestivum* genes

Gene Name	<i>Z. tritici</i> Dutch Isolate IPO 323 β - tubulin	<i>Z. tritici</i> Isolate 396.12 β - tubulin	<i>T. aestivum</i> α - tubulin	<i>T. aestivum</i> GAPDH
Slope	-3.58	-3.42	-2.84	-3.68
Efficiency	1.98 (98%)	1.95 (95%)	1.99 (99%)	2.05 (95%)
Error	0.30	0.40	0.40	0.70
R ²	0.99	0.99	0.97	0.99

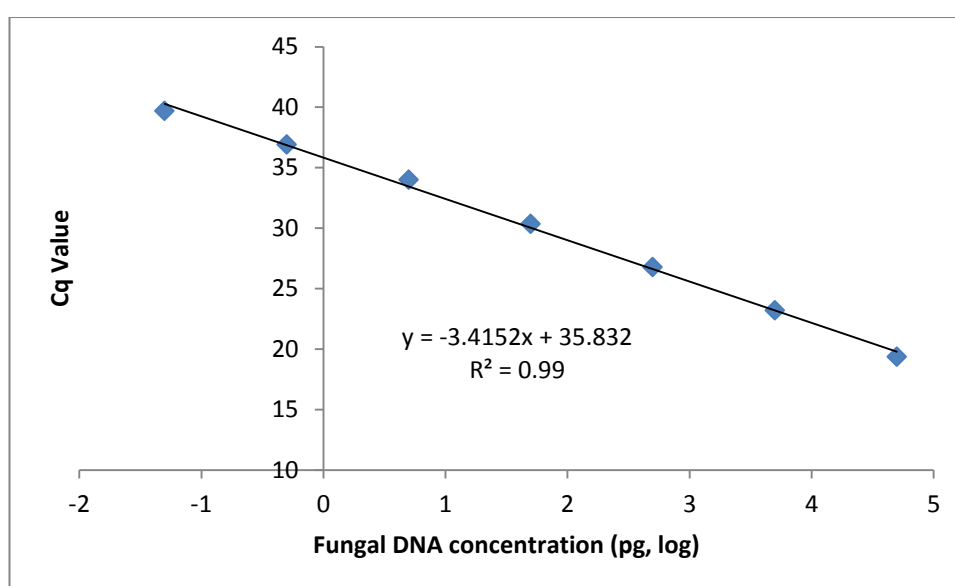


Figure 4.5: Relationship between *Zymoseptoria tritici* β -tubulin strain 396.12 DNA and quantification cycle (Cq) value tested by real-time PCR analysis using a series of *Z. tritici* DNA dilutions (50000-0.05pg/ μ l, each dilutions with three replicates).

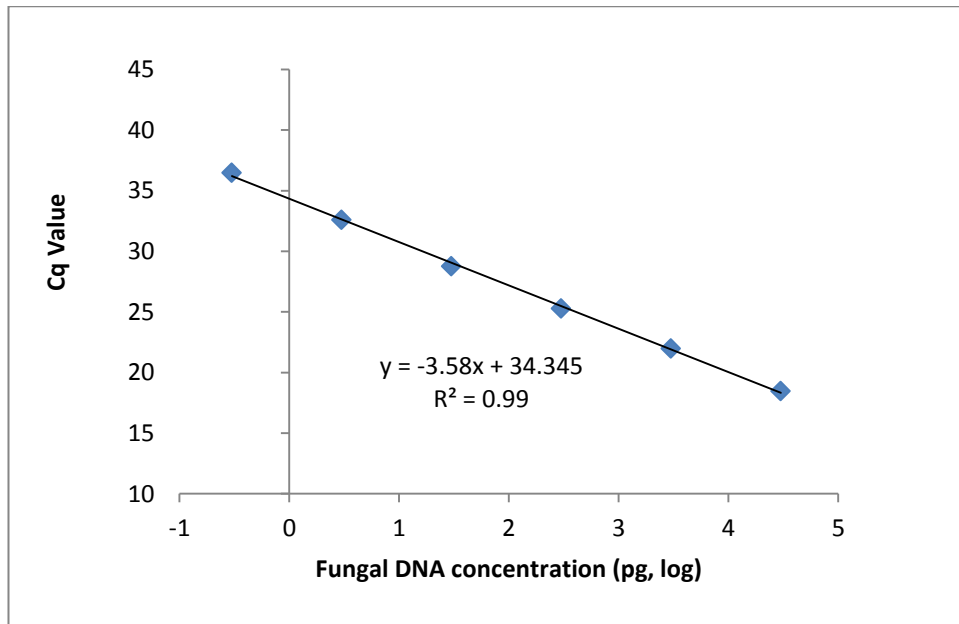


Figure 4.6: Relationship between *Zymoseptoria tritici* β -tubulin strain IPO323 DNA and quantification cycle (Cq) value tested by real-time PCR analysis using a series of *Z. tritici* DNA dilutions (30000-0.3pg/ μ l, each dilutions with three replicates).

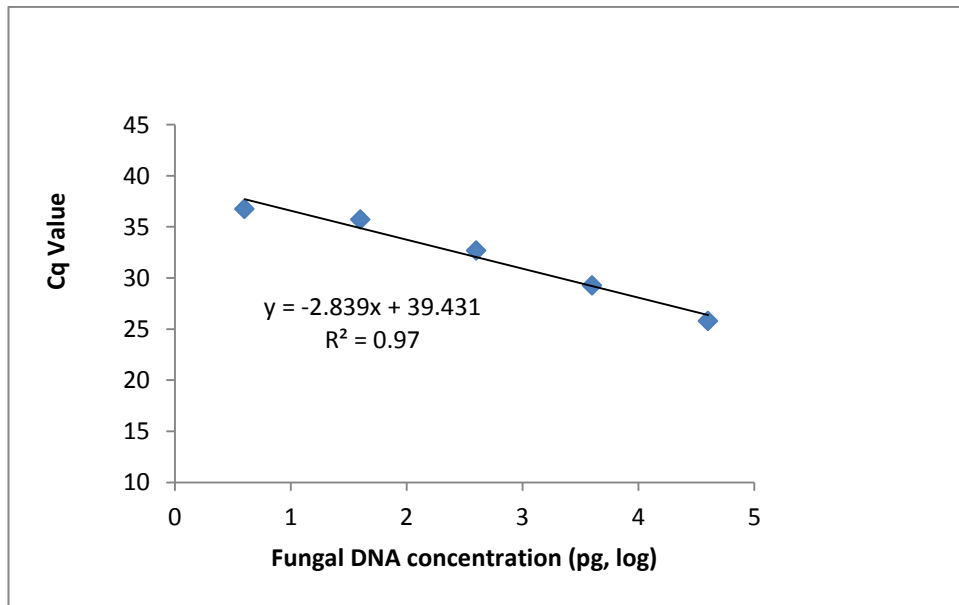


Figure 4.7: Relationship between *Triticum aestivum* α tubulin DNA and quantification cycle (Cq) value tested by real-time PCR analysis using a series of *T. aestivum* DNA dilutions (40000-4pg/ μ l, each dilutions with three replicates).

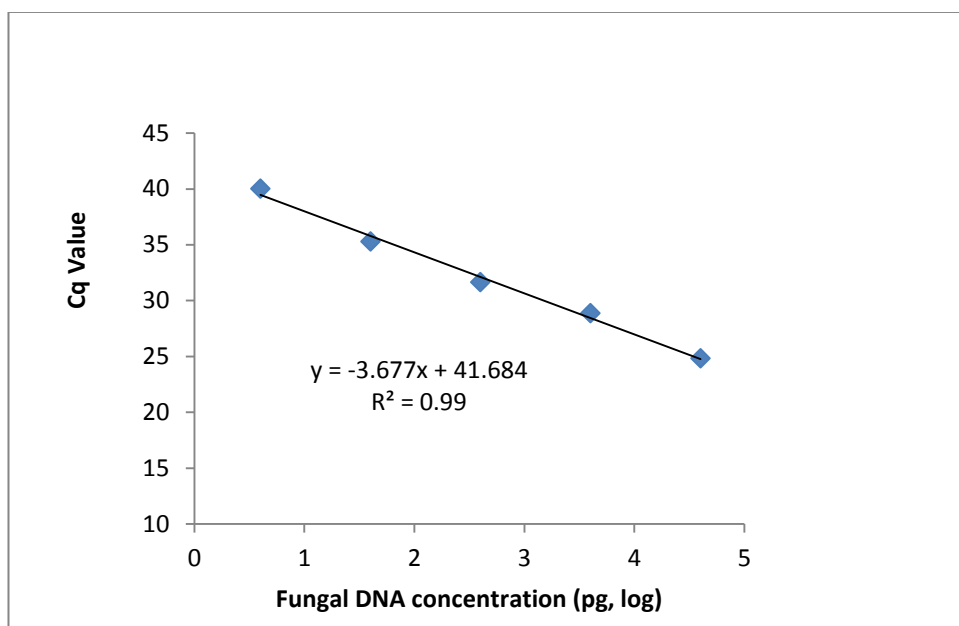


Figure 4.8: Relationship between *Triticum aestivum* GAPDH DNA and quantification cycle (Cq) value tested by real-time PCR analysis using a series of *T. aestivum* DNA dilutions (40000-4pg/ μ l, each dilutions with three replicates).

4.4.2. Glasshouse study

Results from the glasshouse based study indicated that cv. Stigg had high partial resistance to *Z. tritici* in comparison to the highly susceptible cv. Gallant. Cv. Stigg displayed very low levels of the pathogen up to 16 days after inoculation (dai) recording 6.40pg *Z.tritici* / μ g wheat DNA. This then increased almost 10 fold to 61.78pg *Z.tritici* / μ g wheat DNA within another four days at 20 dai (Figure 4.9). In contrast cv. Gallant showed low levels of the pathogens presence up to only 10 dai, when a concentration of 3.87pg *Z.tritici* / μ g wheat DNA was recorded. This concentration then almost doubled within 24 hours, recording 6.35pg *Z.tritici* / μ g wheat DNA at 11 dai. Two days later this concentration tripled to 19.16pg *Z.tritici* / μ g wheat DNA. By 20 dai cv. Gallant's concentration of *Z. tritici* present increased almost 10 fold to 186.75pg *Z.tritici* / μ g wheat DNA, this was three times the concentration of *Z. tritici* present in cv. Stigg on the same day (Figure 4.9). Also the standard errors in cv. Gallant are larger at 16 dai (85.09) and 20 dai (117.64) and in cv Stigg at 20 dai (60.27) (Figure 4.9), this significant variation in pathogen load levels was due to the

biological replicates sampled, i.e. biological rep. 1 at 16 dai had 193.69 pg *Z. tritici* / μg wheat DNA, when rep 2 and rep 3 had 30.42 and 44.30 pg *Z. tritici* / μg wheat DNA respectively. This variation was similar for all biological replicates at 20 dai.

The averaged qPCR results from both time-course trials concurred with the averaged visual disease assessments recorded throughout the study. The relationship between these two datasets is shown in figure 4.10 for cv. Stigg and figure 4.11 for cv. Gallant. Cv. Stigg recorded 0% visual disease symptoms and 1.99pg *Z. tritici* / μg wheat DNA at 10 dai, at 11 dai the pathogen load decreased very slightly to 1.67pg *Z. tritici* / μg wheat DNA and again decrease slightly to 1.35pg *Z. tritici* / μg wheat DNA by 13 dai. At 16 dai the visual disease symptoms had increased to 0.5% and the pathogen load to 6.40pg *Z. tritici* / μg wheat DNA. The pathogen load then increased 10 fold to 61.78pg *Z. tritici* / μg wheat DNA and the visual disease symptoms increased to 7.5% 4 days later at 20 dai (Figure 4.10). Cv. Gallant also recorded 0% visual disease symptoms and a pathogen load of 3.87pg *Z. tritici* / μg wheat DNA at 10 dai. Twenty four hours later at 11 dai the pathogen load had almost doubled to 6.35pg *Z. tritici* / μg wheat DNA and the visual disease symptoms were now 0.5%. At 13 dai the visual disease symptoms had increased to 1% and the pathogen load increased to 19.14pg *Z. tritici* / μg wheat DNA.

Three days later at 16 dai the pathogen load had increased substantially to 108.34pg *Z. tritici* / μg wheat DNA but the visual disease symptoms had only increased to 7.5%. By 20 dai the visual disease symptoms had reached 50% on the leaf surface and the pathogen load within the leaf had increased to 108.75pg *Z. tritici* / μg wheat DNA (Figure 4.11). The visual disease symptoms can also be seen in Figures 4.12 and 4.13.

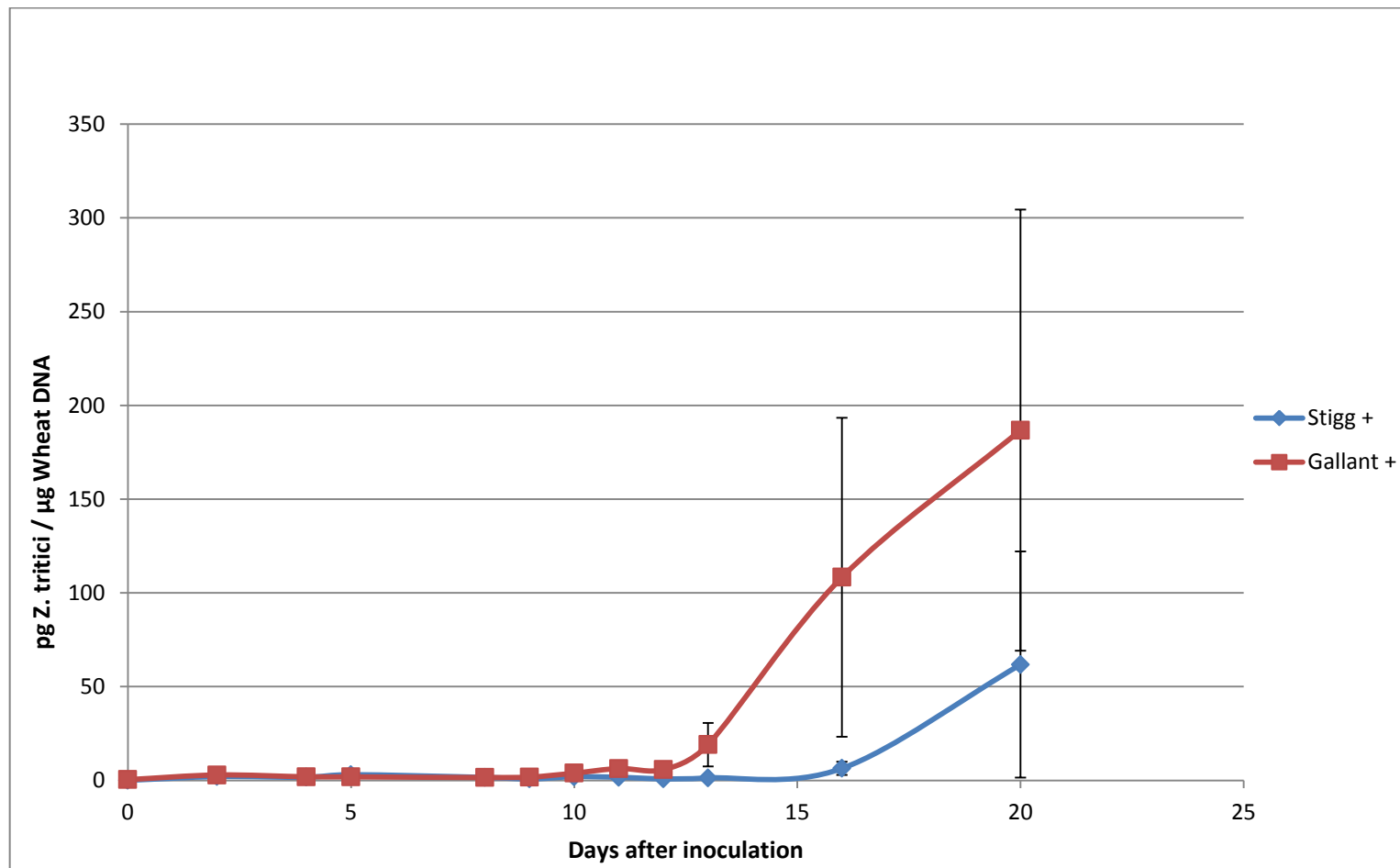


Figure 4.9: Pathogen load in leaves from winter wheat cvs Stigg and Gallant during time course trials in glasshouse study. Error bars indicate standard errors of the mean concentration of *Zymoseptoria tritici* DNA averaged from both trials at each time point.

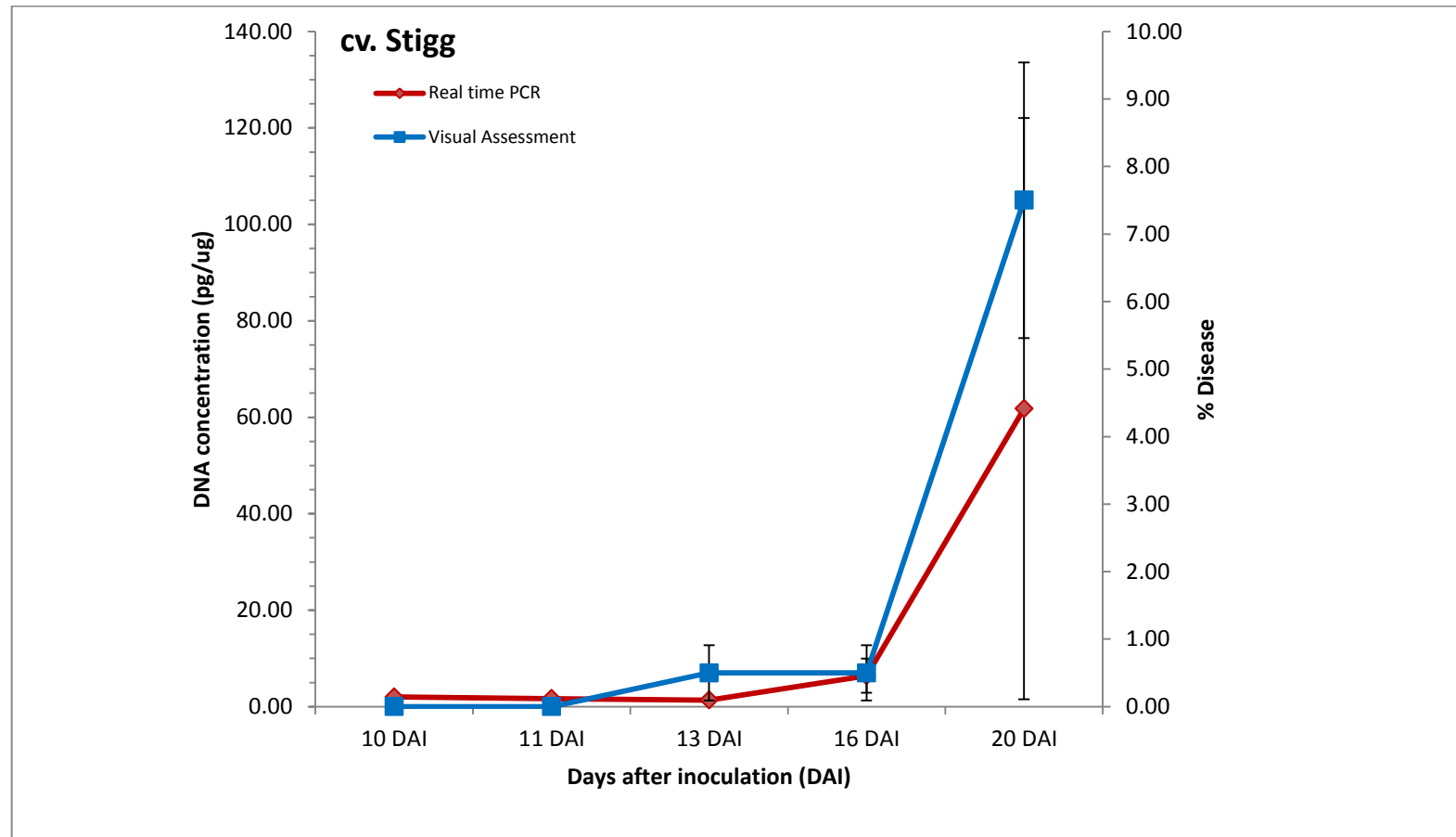


Figure 4.10: Development of *Zymoseptoria tritici* in artificially inoculated seedling plants of winter wheat cv. Stigg in a controlled glasshouse environment assessed by qPCR and a visual disease symptom assessment method 10, 11, 13, 16 and 20 days after inoculation (DAI). Error bars indicate standard errors of the mean concentration of *Z. tritici* DNA averaged from both trials at each time point.

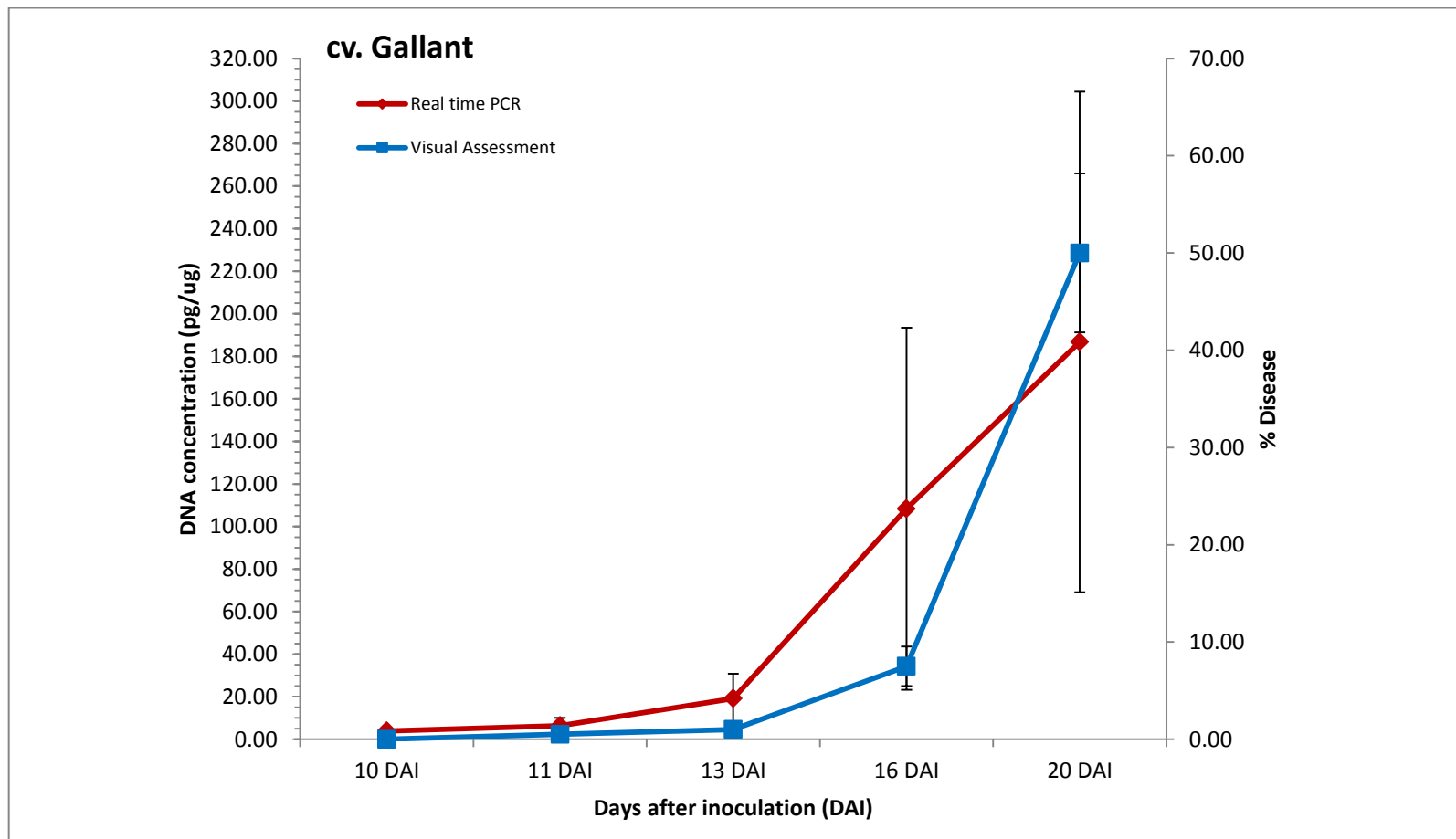


Figure 4.11: Development of *Zymoseptoria tritici* in artificially inoculated seedling plants of winter wheat cv. Gallant in a controlled glasshouse environment assessed by qPCR and a visual disease symptom assessment method 10, 11, 13, 16 and 20 days after inoculation (DAI). Error bars indicate standard errors of the mean concentration of *Z. tritici* DNA averaged from both trials at each time point.

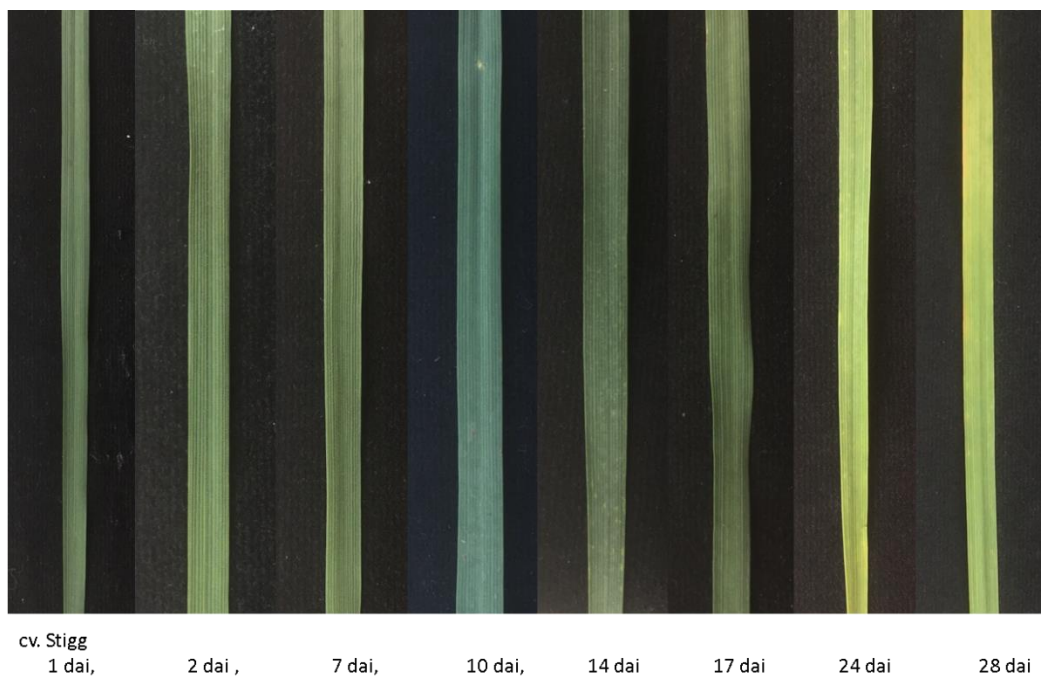


Figure 4.12: Winter wheat cv. Stigg visual disease symptom progression during glasshouse time course trial 2.

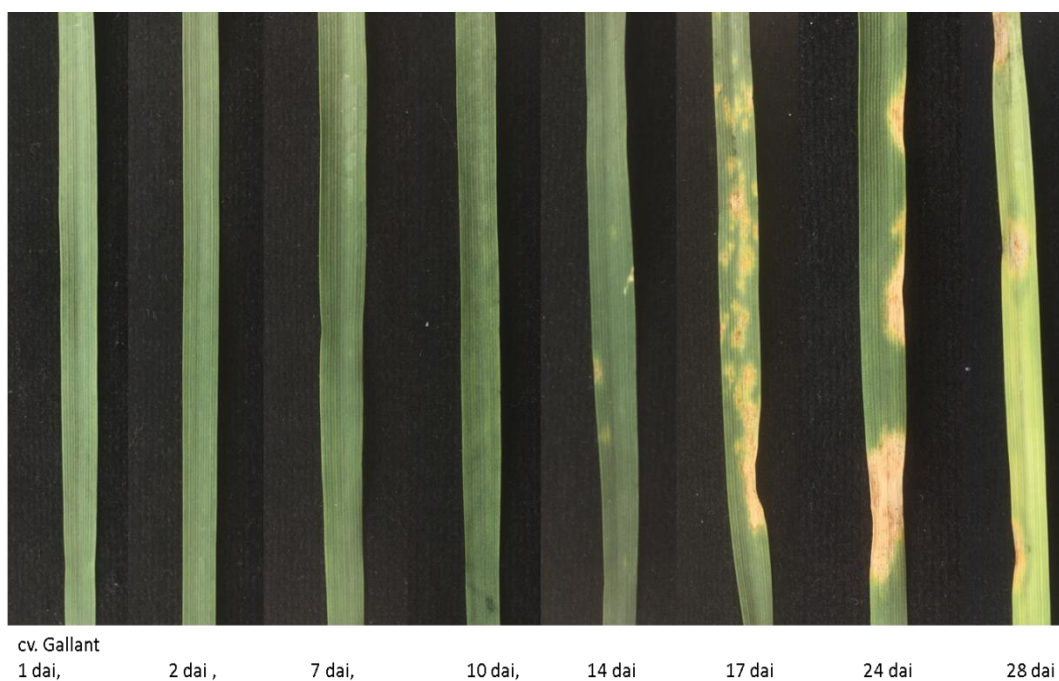


Figure 4.13: Winter wheat cv. Gallant visual disease progression during glasshouse time course trial 2.

4.4.3. Field study

Results from the field study indicated that all seven cultivars showed very low levels of the pathogens presence at the first sampling date (08 May 2015). By the second sampling date (26 Jun 2015) cvs. Gallant and Gator had very high susceptibility to *Z. tritici* infection (7418.15pg/μg and 9481.32pg/μg, respectively, all units are in pg *Z. tritici* / μg Wheat DNA), in comparison to the other 5 cvs (Stigg: 1927.82pg/μg, Dunmore: 74.11pg/μg, Croft: 0.07pg/μg, JB Diego: 76.52pg/μg, Kielder: 621.12pg/μg). However, cv. JB Diego had a significant standard error due to biological replicate 5 registering a Cq value of 27 cycles and biological replicates 1-4 registering Cq values of 40 cycles. This equates to reps 1-4 having no *Z. tritici* present (concentration ranging from 0.05pg to 0.07pg *Z. tritici* / ng Wheat DNA) at this time and rep 5 having a concentration of 382.35pg *Z. tritici* / ng wheat DNA (Figure 4.14). At the third and final sampling date (08 Jul 2015) cvs Gallant and Gator remained the most susceptible cultivars of the seven (323921.32pg/μg and 19399.59pg/μg, respectively). Cultivars Dunmore, Croft, JB Diego, and Kielder recorded increased concentration of *Z. tritici*, all >4000pg *Z. tritici* / μg Wheat DNA, but cv. Stigg recorded a decrease of 1119.78pg/μg between the second and third sampling dates, indicating a concentration of just 808.04pg *Z. tritici* / μg Wheat DNA by the final sampling date (Figure 4.14).

A visual representation of the % disease progression visual assessments from Chapter 3 and, pg *Z. tritici* / μg wheat DNA from the qPCR analysed data was plotted against the three sample / assessment dates for each of the 7 winter wheat varieties in the study (Figures 4.15 – 4.21). The sampled leaves for the qPCR analysis were not the same leaves visually assessed in the field, as the visual assessments were carried out on the same 2nd leaf and flag leaf of the same tiller throughout the growing season and therefore could not be sampled for further analysis. Figure 4.15 describes when cv. Stigg had 0% visual disease symptoms on the 8th May 2015; its sampled leaf tissue contained 0.03pg *Z. tritici* / μg wheat DNA. On the 26th June 2015 the visual disease symptoms had increased to 7.91% and the sampled leaf tissue contained 1927.82pg *Z. tritici* / μg wheat DNA. On the final sampling date 7th July 2015 cv. Stigg showed an increase in visual disease symptoms (10.60%) but a decrease in pathogen DNA

present in the leaf (808.04pg *Z. tritici* / µg wheat DNA). Cv. Croft displayed a similar trend of 0% visual disease and 0.08pg *Z. tritici* / µg wheat DNA on the 8th May 2015, then a slight decrease in pathogen load 0.07pg *Z. tritici* / µg wheat DNA but an increase in visual symptoms (9.57%) by the 26th June 2015. Both parameters then increased by the final sampling date 8th July 2015 (24.80% disease progression and 4292.23pg *Z. tritici* / µg wheat DNA), (Figure 4.16). This was most probably due to the leaves sampled and the leaves visually assessed, being different winter wheat plants and therefore was an indicator of the variance of *Z. tritici* disease levels throughout the field study site. Figure 4.17 shows cv. Dunmore displaying 0% visual disease symptoms on the 8th May 2015 but had a pathogen load of 0.12pg *Z. tritici* / µg wheat DNA in its sampled leaf tissue. During the following 49 days cv. Dunmore showed a slow increase in visual disease symptoms reaching 9.96% (74.11pg *Z. tritici* / µg wheat DNA) on the 26th June 2015 and then increasing within 12 days to 30.70% (4990.11pg *Z. tritici* / µg wheat DNA). Cv. JB Diego showed a similar trend to cv. Dunmore showing 0% visual disease and 3.96pg *Z. tritici* / µg wheat DNA on the first sampling date, then increasing slowly to 4.89% visual disease and 76.52pg *Z. tritici* / µg wheat DNA on the second sampling date and finally increasing to 11.5% visual disease and 8365.30pg *Z. tritici* / µg wheat DNA within the final 12 days of assessments (Figure 4.18). Cv. Kielder showed 0.12pg *Z. tritici* / µg wheat DNA on the 8th May 2015 when it had 0% visual disease, this increase to 621.12pg *Z. tritici* / µg wheat DNA and 7.13% visual disease by the 26th June 2015 and finally reached 4934.98pg *Z. tritici* / µg wheat DNA and 25.60% visual disease by the 8th July 2015 (Figure 4.19). Cv. Gator behaved in a similar manner in regards to the rate of disease progression however pathogen load in the sampled leaf tissue was significantly higher. On the 8th May 2015 cv. Gator showed 0% visual disease and 0.05pg *Z. tritici* / µg wheat DNA, but by the 26th June 2015 this had significantly increased to 17.42% visual disease and a pathogen load of 9481.32pg *Z. tritici* / µg wheat DNA. By the final sampling date (8th July 2015) the visual disease symptoms had increased to 37.10% and the pathogen load to 19399.59pg *Z. tritici* / µg wheat DNA (Figure 4.20).

Finally the most susceptible winter wheat cv. Gallant displayed the highest percent visual symptoms and also the highest pathogen load (pg/ μ g). On the 8th May 2015 cv. Gallant performed as the other 6 cvs., showing 0% visual disease and a pathogen load of 0.03pg *Z. tritici* / μ g wheat DNA. By the 26th June 2015 these values had increase significantly to 25.42% visual disease and 7418.15pg *Z. tritici* / μ g wheat DNA. On the 8th July 2015 the visual disease symptoms on cv. Gallant had increased almost 3 fold to 68.5% and the pathogen load had increased 4 fold to 323921.33pg *Z. tritici* / μ g wheat DNA (Figure 4.21).

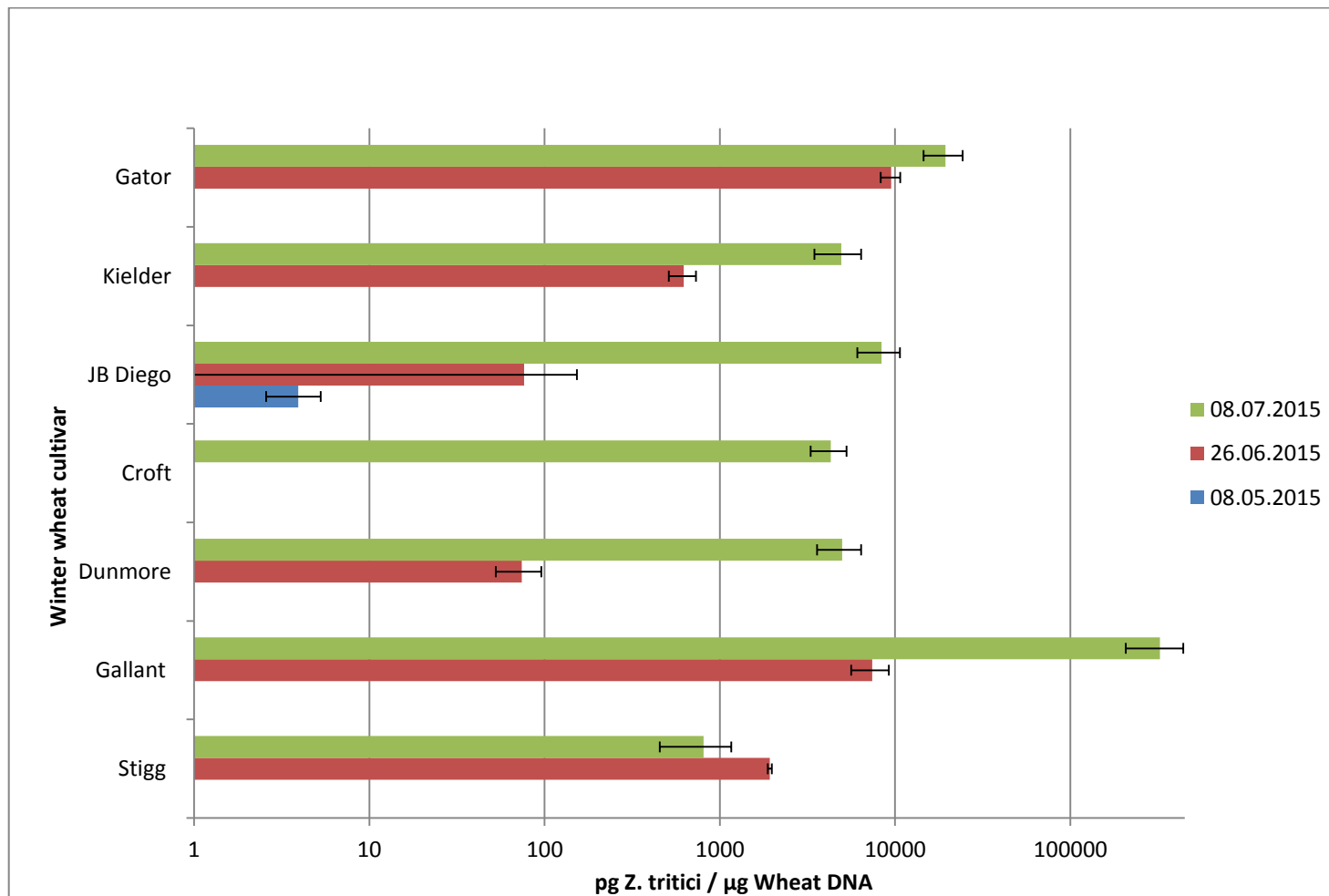


Figure 4.14: Pathogen load (pg *Z. tritici* / µg wheat DNA) in leaves sampled from Oak Park field trial 2015, pg *Z. tritici* / µg Wheat DNA plotted in logarithmic scale.

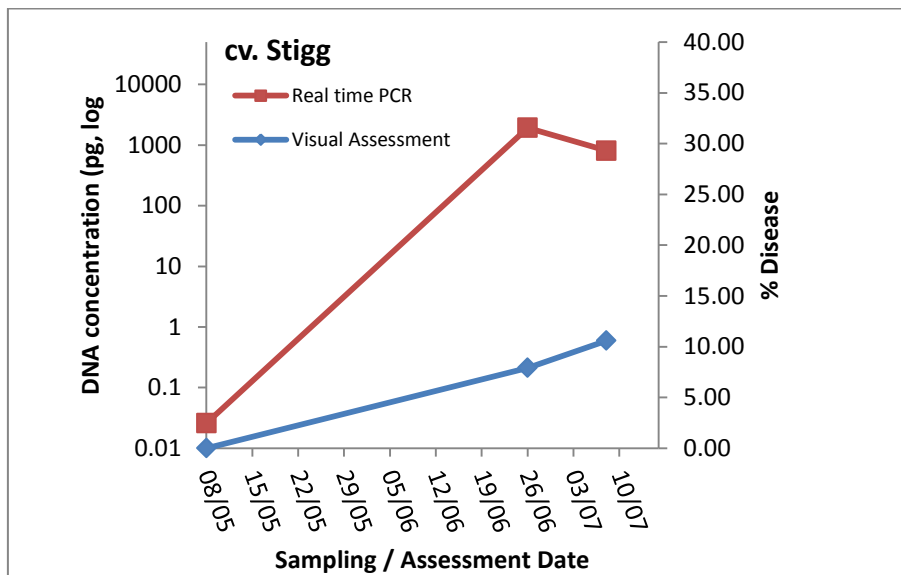


Figure 4.15: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. Stigg assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

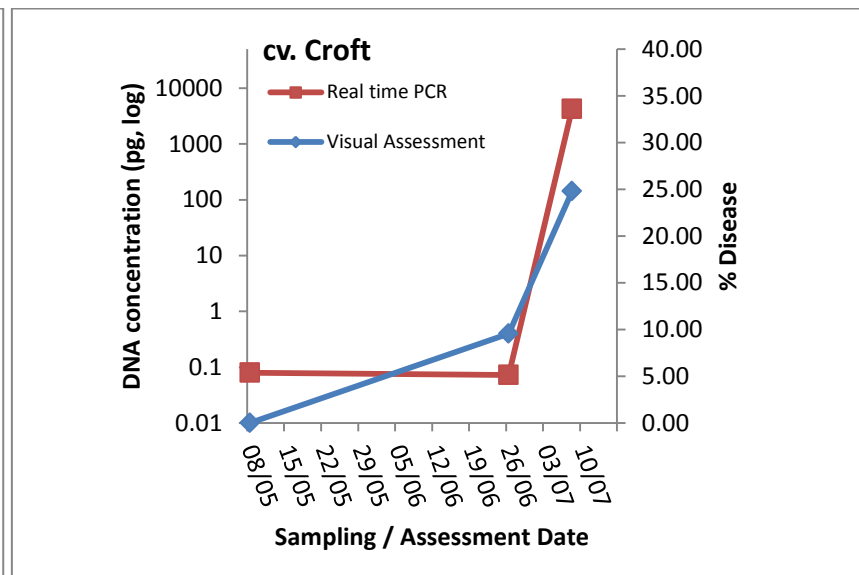


Figure 4.16: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. Croft assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

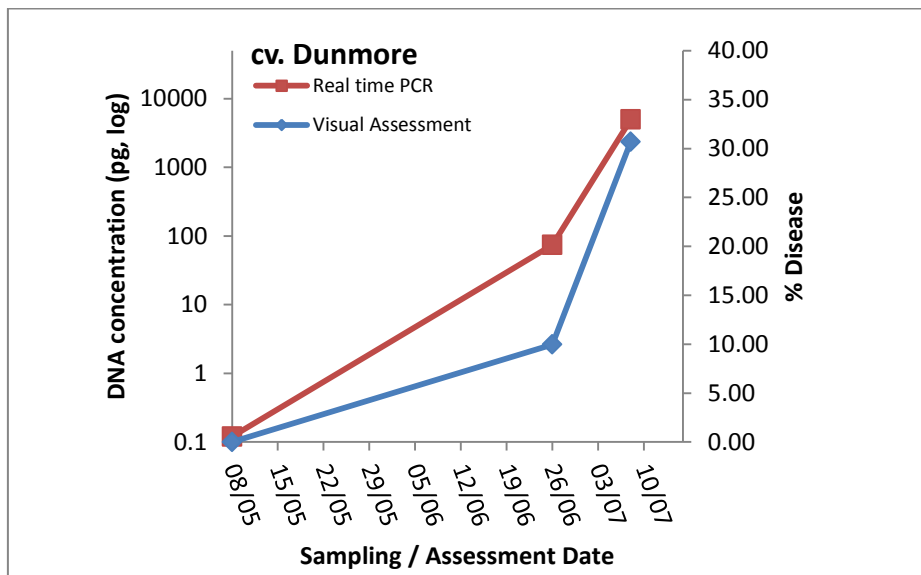


Figure 4.17: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. Dunmore assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

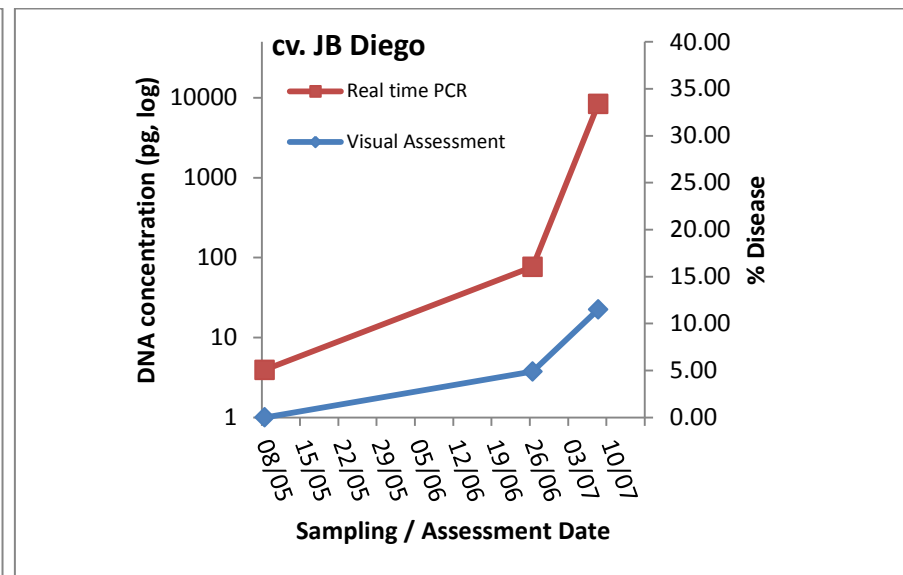


Figure 4.18: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. JB Diego assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

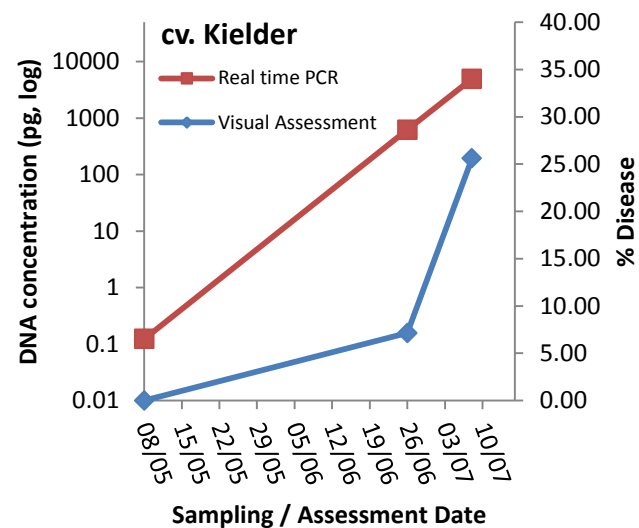


Figure 4.19: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. Kielder assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

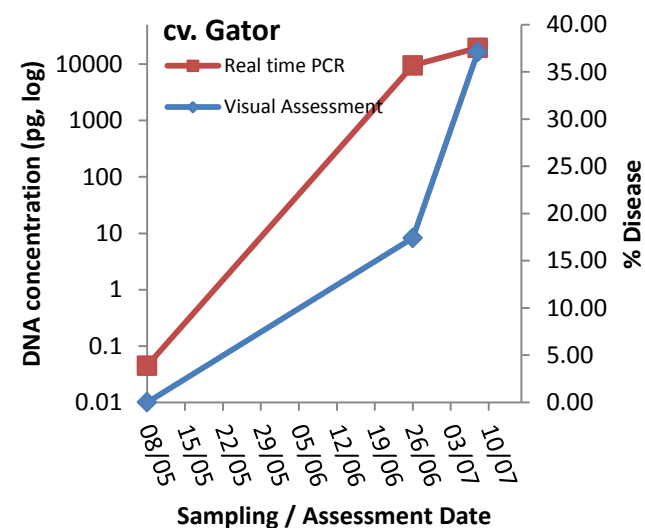


Figure 4.20: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. Gator assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

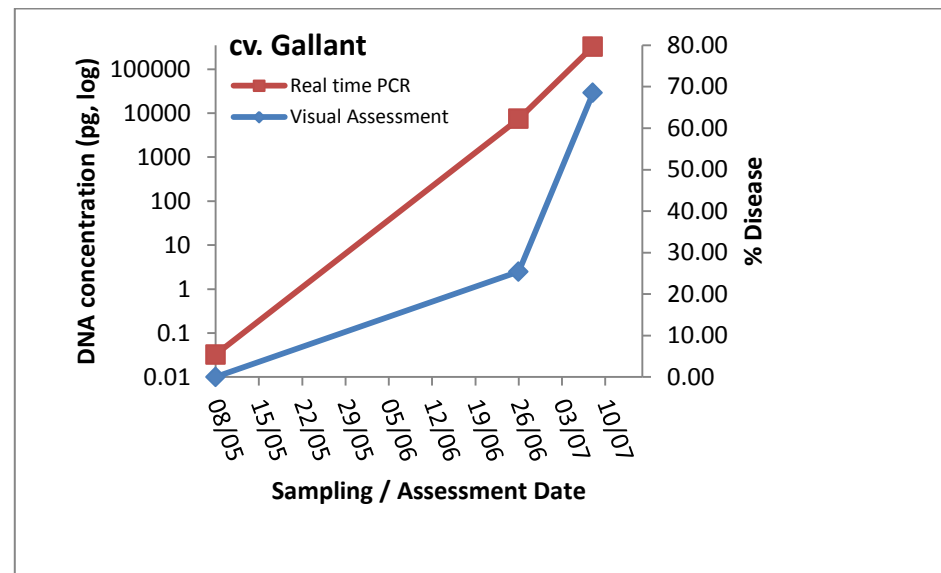


Figure 4.21: Development of *Zymoseptoria tritici* in naturally infected field samples of winter wheat cv. Gallant assessed by qPCR and a visual assessment method on the 8th May, 26th June and 8th July 2015.

4.5. Discussion

Quantitative PCR is well suited to the detection of hemibiotrophic pathogens such as *Z. tritici* which have no visual disease symptoms until infection is well established within the host plant and has previously been shown to detect pre-symptomatic disease (Guo *et al.*, 2006).

The main objectives of this study was to establish a qPCR assay following Guo *et al* (2006) and apply this assay to quantify the pathogen load inside the host leaf tissue at set time-points in an effort to better understand how the pathogen in question infects and establishes itself within the host during the LP. In order to relate the concentration of *Z. tritici* DNA to the visual disease assessments, the naturally infected field leaf samples were also subjected to the qPCR assay. The assay showed a very tight (negative) correlation between PCR cycles and fungal growth, i.e. the Cq value gives a precise measurement of fungal DNA concentration.

Technical issues were encountered after the original optimised qPCR assay was used to quantify the fungal biomass of *Z. tritici* IPO323 in the glasshouse. The assay was run on the samples numerous times and consistently returned negative results for the presence of *Z. tritici*. The recorded visual assessment data and photographs contradicted the returned negative qPCR data. With this in mind, further research was carried out into the two *Z. tritici* target gene sequences which highlighted the slight differences in the target sequences. This was a significant amount of re-work in designing new probe and primers for the new *Z. tritici* target gene and also the re-optimisation of the qPCR assay to ensure the assay could then be used for the glasshouse sample analysis. With this in mind, it is also possible that the primers designed for the testing the field sample may not have detected other *Z. tritici* field isolates which may have been present in the samples. Therefore, further research is required using a broader range of primers to test for the presence of *Z. tritici* DNA in field samples.

4.5.1. Glasshouse study

The optimised qPCR method indicates the latent period of *Z. tritici* in each of the winter wheat varieties and shows that cv. Stigg has a longer LP than cv.

Gallant. The 3 biological reps sampled at each time point did not have equal % of visible disease. This is apparent in the resulting dataset and can be seen in figure 4.9 when cv. Gallant recorded 6.3575pg *Z. tritici* / µg wheat DNA at 11 dai and recorded 5.77pg *Z. tritici* / µg wheat DNA at 12 dai. This decrease is most likely due to one of the biological replicates harvested and quantified containing a lesser quantity of fungus present in its leaf tissue and therefore was skewing the data when the 3 biological reps were averaged for data analysis. This was also highly evident from the results of the qPCR analysis for 1dai, 7dai, 14dai, 17dai, 24dai and 28dai and therefore these time-points were removed from the final dataset when plotting the graph to minimise skewing of the graph (Figure 4.9).

Further studies require a larger, more precise in depth analysis of *Z. tritici* DNA concentration during the latent period and also a wider range of partially resistant and susceptible winter wheat varieties included in the analysis. Also for future studies in an artificially inoculated glasshouse study, an increase in biological reps within the time course trial would reduce the standard errors, for a more accurate dataset.

4.5.2. Field study

There is a clear trend between the visual disease assessments and *Z. tritici* DNA presence in the sampled leaves for cvs Stigg and Gallant and the results were as expected in regards to partial resistance and susceptibility to *Z. tritici*. Winter wheat cv. Stigg showed the lowest concentrations of pg *Z. tritici* / µg wheat DNA (808.04 pg/µg) and also percent disease progression (10.60%) and cv. Gallant showed the highest concentrations of pg *Z. tritici* / µg wheat DNA (323921.33 pg/µg) and percent disease progression (68.5%) at the final sampling / assessment date. The wide range in *Z. tritici* concentration present in the sampled leaves of the different varieties at the 2nd sampling date (26.06.2015) is interesting and is indicative of how the individual varieties react

differently to infection of the fungal pathogen *Z. tritici*. Also the decrease of pg *Z. tritici* / μg Wheat DNA in cv. Stigg between the 2nd and 3rd sampling dates is most probably not a biological phenomenon, but more likely an artefact of sampling. However, it is possible that this decrease in pathogen load is indicative of the variance in disease levels throughout the five biological replicates in an uncontrolled field environment.

For future studies in a naturally infected field environment the visual disease assessments should ideally be taken from the same leaf as used for the qPCR analysis, and also increasing the number of sampling dates should give more robust estimates of the duration of the latent period and of the rate of pathogen growth once disease is established (i.e. once lesions are visible) between visual disease symptoms and qPCR outputs.

Figure 4.15, describing the development of visual disease symptoms and pg *Z. tritici* / μg wheat DNA in cv. Stigg may be highlighting a new biological insight in a natural field environment. Although cv. Stigg appears resistant based on a long LP because the visual assessments were showing no significant disease progression over the three assessment dates, the fungal biomass concentration was increasing in a similar manner to other cultivars. However this data was recorded in only one of the three growing season, therefore further trials are needed to obtain any definite conclusions.

Chapter 5: General Discussion and Conclusion

The goal of this research was to investigate the rate of fungal growth during the *Z. tritici* LP and identify the distribution of LP's across a range of winter wheat cultivars representing high partial resistance, moderate resistance and high susceptibility to this fungal pathogen which devastates winter wheat crops worldwide including in Ireland and the UK. To initiate this, a preliminary glasshouse study was conducted to identify winter wheat cultivars with contrasting degrees of partial resistance and susceptibility to STB (Chapter 2). Seven of these cultivars cv. Stigg (high partial resistance), cv. Dunmore (high partial resistance, cv. JB Diego (moderate resistance), cv. Croft (moderate resistance, cv. Oakley (moderate resistance) cv. Kielder (moderate resistance), and cv. Gator (high susceptibility) were initially carried forward to the field study. After the first year of the field study, cv. Oakley was lost to yellow rust and replaced with cv. Gallant (high susceptibility) in the subsequent field experiments.

The LP is an important phase in the lifecycle of *Z. tritici* (Dean, 2012) and its length is directly affected by environmental conditions such as temperature, humidity and inoculum pressure (Viljanen-Rollinson *et al*, 2005). The field study (Chapter 3) investigated the approximate length of the LP of the seven selected winter wheat cultivars under different environmental conditions. By investigating the approximate LP length of winter wheat varieties under different environmental conditions, this data may further assist in the prediction of *Z. tritici* events. Latent period data can be used to optimise the timing of fungicide applications because a fungicide may only provide eradicated control for approximately half the LP length, but if the infection is too far into the LP then no quantity of fungicide will control the fungal pathogen (HGCA, 2015). This means that the optimum spray timing will vary for different winter wheat varieties.

The results of the field trial neither proved or disproved my initial hypothesis that the LP of *Z. tritici* in the winter wheat cultivars would follow a similar trend in the natural field environment as in the controlled glasshouse environment. Some cultivars showed similar LP's whilst other showed varying LP's. Cultivar Stigg (high partial resistance) recorded the longest LP in both environments (24.2

days in the glasshouse, 27.6 days in Waterford (high inoculum pressure), 25.6 days in Oak Park (medium inoculum pressure), no LP data available for Norwich). Cultivar JB Diego followed cv. Stigg recording the second longest LP (21.1 days in the glasshouse, Waterford recorded an LP of 24.8 days and Oak Park recorded an LP of 21 days). Although cv. Gallant (most susceptible) recorded the shortest LP (12 days) in the glasshouse, it recorded an LP of 21.8 days in Waterford and 20.4 days in Oak Park. As the LP data was only collecting during the 2014-2015 growing season, further research into LP is required in a natural field environment. Also a more definitive measurement of LP in a natural inoculum environment is required in further research, as the field trials are subjected to varying and potentially continuous inoculum load. The results of the field evaluation demonstrate that environmental conditions strongly influence the early responses of wheat to infection by *Z. tritici* which reinforces previous research (Viljanen-Rollinson *et al*, 2005). They also emphasise the importance of investigating further the biology of this complex pathosystem. Although the field study sites were geographically different and had different disease inoculum pressures, they showed similar trends over the three growing seasons. All recorded data was indicative that cv. Stigg remains the highest partially resistant winter wheat cultivar at all three field study sites.

Although cv. Stigg had the longest LP it was removed from the HGCA recommended lists in the UK due to the rapid and severe “breakdown” of its resistance to the new strains of yellow and brown rust, which first appeared in 2011. In Ireland yellow rust is more common and so cv. Stigg was removed from the DAFM recommended lists for the same reason.

For this reason, it is desirable to study a wide variety of winter wheat cultivars for *Z. tritici* resistance across geographically different field environments which represent varying inoculum pressures, but also to record if the various cultivars are susceptible to other diseases for example, cv. Oakley getting severely diseased by yellow rust in the first year of the field study. This will enable agronomists or farmers to choose a winter wheat cultivar best suited to their geographical location based on disease resistance and financial return on yield. The use of glasshouse trials can complement a field trial by enabling data collection from both a controlled environment and a natural uncontrolled environment. Resulting datasets can then be analysed for correlations between

varieties' LP lengths in controlled and uncontrolled environments. In this study there were similar varietal LP lengths in the glasshouse and the medium inoculum pressure site in Oak Park, Carlow. But data also showed significant differences in varietal LP length between environments, therefore further research is required to understand the influence of the environment on LP and thus disease development in diverse varieties.

The quantification of the rate of fungal growth in seven winter wheat cultivars from the field study (Chapter 3) and also cv. Stigg (most resistant) and cv. Gallant (most susceptible) from the glasshouse study (Chapter 4) during the *Z. tritici* latent period by qPCR allowed the fungal biomass in the leaves sampled to be quantified in pg *Z. tritici* / µg wheat DNA. This data then allowed for the relationship between DNA concentration and visual disease symptoms to be graphed as a visual representation of how the concentration of pg *Z. tritici* / µg wheat DNA clearly correlates with the expression of visual STB disease symptoms. This gives a further insight into the genetic responses of the various winter wheat cultivars to infection of *Z. tritici* during its LP by providing a solid dataset and starting point for future, more in depth research into the genetic interactions between the host's defences and the pathogen during the crucial period of infection.

The work reported in Chapter 3, sections 3.2, 3.3 and 3.5 has been published in the paper by J. G. Hehir, C. Connolly, A. O'Driscoll, J. P. Lynch, J. Spink, J. K. M. Brown, F. Doohan and E. Mullins. Temporal and spatial field evaluations highlight the importance of the presymptomatic phase in supporting strong partial resistance in *Triticum aestivum* against *Zymoseptoria tritici*. Plant Pathology, 2017.

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